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Hybridization and embryology of Paspalum dilatatum Poir

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NOTE TO USERS

HYBRIDIZATION AND EMBRYOLOGY OF *PASPALUM DILATATUM* POIR.

by

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INTRODUCTION

Dallis grass (Paspalum dilatatum Poir.) has proven to be one of the most valuable pasture and hay plants for the Southeastern States. It has more desirable characteristics than the other commonly used pasture grasses, Bermuda (Cynodon dactylon (L) Pers.) and Carpet (Axonopus compressus (Swartz) Beauv.). It produces many basal leaves which, following grazing or drought, make a rapid recovery. It is the first grass to begin growth in the spring, makes continuous growth during warm weather, is not injured by moderate frosts, and is the last grass to become dormant in the fall. Its bunch habit of growth makes it more adapted to associated growth with pasture legumes and also prevents it from becoming a pest in fields that are to be rotated. It is more tolerant to excessive soil moisture and, at the same time, more drought and heat resistant. It grows well on all soils except the extremely sandy ones, and is the basic summer grass recommended for permanent pastures in Mississippi.

Undesirable characteristics of Dallis grass are low seed production and complete susceptibility to an ergot, Claviceps paspali (Stevens and Hall). The total lack of disease resistance nullifies any selections toward high seed production. The value of such selections is in their use as parents in hybridization.

Paspalum urvillei (Steud.) and P. malacophyllum (Trin.) are respectively resistant and immune to ergot, but are not able to persist under pasture conditions. The incorporation of their disease resistance and disease immunity into P. dilatatum would improve the pasture qualities of the latter and permit the selection for seed production. Attempts to make hybrids with these two species, under field and greenhouse conditions, has previously met with repeated failures. Hybrids are easily obtained between P. malacophyllum and P. urvillei. Differences in the time of flowering, pollination, and the short time the pollen remains viable have accounted for the failures of attempted hybridization of P. dilatatum with these two species.

The increasing importance of P. dilatatum as a pasture plant warrants more detailed studies as to its cytology, morphology, and life history. Information from such studies, particularly the cytological phases, may have a direct application to problems relating to the development of improved strains through plant breeding. Such studies might reveal causes of failures of attempted hybridization and the low per cent of seed setting.

As seed production is the factor limiting a more widespread use of this plant, it is desirable to obtain information regarding seed development. The object of one section of this study was to trace the developmental morphology of the embryo in an effort to determine not only the manner of development but also the order and rate of

development of the various parts. A study of the normal development of the embryo in this economically important species would serve as a starting point for investigations of the various types and abnormalities in P. dilatatum and in other species.

A review of the literature shows that many uses are being made of the artificial germination of the pollen of a wide variety of plants. Some evidence has indicated a correlation between the percentage of good pollen, or the percentage of pollen germination and the ultimate fertility of plants. If such a correlation were established in Paspalum dilatatum it would permit the ready estimation of the more fertile selections and would reduce the amount of time required, permitting the handling of a larger population per season. It would also permit the selection of the more fertile plants before ergot attacked. Fertile selections would prove valuable for seed production in years when ergot infection was not total.

The purpose of this investigation was (a) to determine the conditions controlling flowering and pollination that would facilitate hybridization, (b) to trace the developmental morphology of the embryo, and (c) to determine the value of artificial pollen germination as a basis of selection for improved seed production. In order to achieve objective (c) it was necessary to study the conditions controlling pollen germination and to correlate such germination with seed set.

REVIEW OF LITERATURE

The susceptibility of *Paspalum dilatatum* to *Claviceps paspali* has been known for many years (5, 49, 62). Brown and Ranck (19) described the life history of this ergot and pointed out its poisonous properties under Mississippi conditions. Cattle and small animals were fed the sclerotia and developed the characteristic nervousness shown by cattle grazing in pastures where the infected grass was growing. The cattle were not killed by the poison directly but were rendered helpless by its influence and perished due to lack of food and water. If they were found in the early stages and taken from the pasture and given food and water, they recovered in a few days. The practice of clipping the pasture twice during the grazing season prevented poisoning. Brown and Ranck also point out that the fungus attacks the female portion of the florets and grows as a parasite in them and that the mycelium occupies the space between the glumes of the spikelet. Only sclerotia are capable of causing the characteristic nervousness.

The active principle of this ergot has recently been isolated by Geiger and Barrentine (35). They find that it is an amorphous compound and give the lethal dosage as 22.5 mg. per kg.

The degree of susceptibility of *Paspalum dilatatum* to ergot in Mississippi was not fully recognized until the initiation of the

Forage Crop Breeding program in 1936 (14). The progeny of disease-free plants selected from long-established (25 to 40 years) pastures throughout the state showed no resistance. Plants from seed obtained from many parts of the world and from all of the southeastern states have likewise shown no resistance. The numbers of selections and introductions tested (all infected by natural inoculation) during the five-year period, 1936-41, have led to the conclusion that there is no resistance in Paspalum dilatatum to Claviceps paspali (15).

Dallis grass bears its "seed", or caryopsis, enclosed in glumes which do not free the seed upon threshing. The seeds are very small and the distinction between seed and empty florets is therefore extremely difficult. Crushing 100 or more florets from each plant with tweezers, or by cutting, are laborious and costly methods for the determination of the percentage of good seed in any large number of plants. The blower method, as described by Brown and Porter (18), used for purity analyses has been used to determine per cent seed.

The percentage of seed in 10,000 spaced (36" x 36") plants was determined by this method. The seed production of this population ranged from 8 to 83.5 per cent. Germination tests on purity samples showed a small, but not significant, negative correlation with the per cent seed or purity (14).

This lack of agreement was found to be due to the high per cent of florets containing ergot. In making purity determinations such

ergot as can be readily picked out (Figure 1) is removed. Considerable numbers of florets, however, contain just enough ergot to withstand the air blast (Figures 2-3) but not enough to enable their elimination by screening, blowing, or by hand. These florets have the same appearance and approximately the same weight as florets containing seed (Figures 4-5). The small negative correlation indicates that the blower method determines per cent ergot infection rather than per cent seed. Selection for seed production by the blower method is therefore considered unreliable and such selection will necessarily depend upon germination or cutting tests.

Total susceptibility to ergot, however, nullifies the value of selections made solely for seed production, regardless of the method of selection. Hybridization is necessary before the seed production of Paspalum dilatatum can be materially improved. The small florets make emasculation almost impossible under natural conditions of flowering. Controlled flowering would eliminate this difficulty as well as those arising from the differences in time of flowering and pollination between the three species. Conditions permitting perfect emasculation and the shedding of pollen at the same time would result in the hybridization of P. dilatatum with P. malacophyllum and P. urvillei.

The inflorescence of grasses varies (38) from the simplest form, the typical raceme (Pleuropogen), through the spike-like raceme (Digitaria), the spike (Hordeae), the panicle (Poa) to the compound

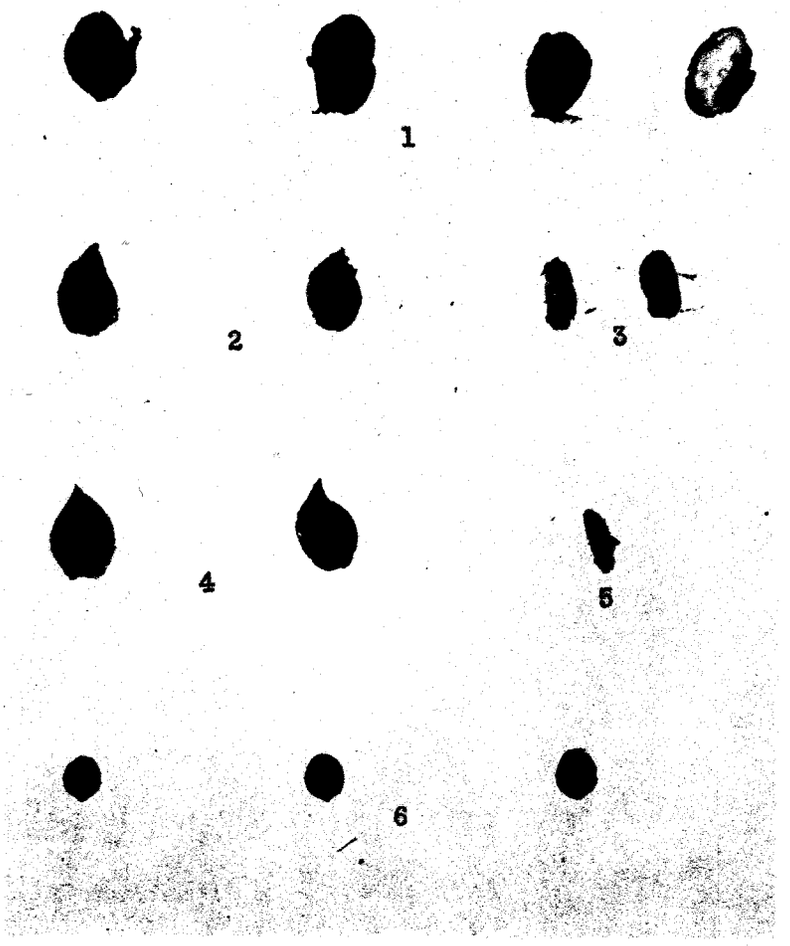


PLATE I

Figure 1. Spikelets removed in purity determinations.
Figure 2. Spikelets containing ergot and withstanding air blast.
Figure 3. Cross-section of 2.
Figure 4. Spikelets containing seed.
Figure 5. Cross section of 4.
Figure 6. Seed free from glumes.

type (Andropogon virginius). The panicle, although quite variously modified, is the most common form. The panicle may be open or diffuse as in Panicum capillare, contracted as in millet, or compact and cylindric as in timothy.

Regardless of the type of inflorescence, Vinall and Hein (91) state that: "flowering begins near the apex of the inflorescence and progresses more or less regularly toward the base. In the spikelet the reverse is true; the basal florets open first, followed in regular order by those above." Fruwith (34), who conducted some rather extensive studies on grass flowering at Hohenheim and Waldhof in Germany with eight genera and thirteen species of grasses, and Stephens and Quinby (85) working with sorghums in Texas concur in this view. Wolfe (96), states that in orchard grass (Dactylis glomerata L.): "As a rule the first flowers to bloom on a head are in the apex, but there is apparently no definite order in other parts of the head." He does, however, show a very definite order in the spikelet: "In a single spikelet the first or lowest flower blooms first followed in order by the second, third, etc., at intervals of about 30 hours."

Grasses extrude their stamens and liberate pollen most abundantly in the early morning or forenoon. Beddows (13) reports this is an almost universal rule although the period may be delayed and prolonged by atmospheric conditions. Jenkin (41) shows that the greater part of blooming takes place in the forenoon. Wolfe (96) observed that 76.9 per cent of orchard grass flowers bloomed from sunrise to noon

with the maximum occurring from 8 to 9 a.m. Some grasses, however, flower in the afternoon or at night.

Although the majority of grasses flower in the forenoon, there is in several grasses a secondary, less intensive flowering period in the afternoon. Fruwirth (34) shows secondary flowering that was less intensive in 7 of the 8 genera studied, and which lasted only 1 or 2 hours. Li, et al. (50) and Rangaswami Ayyangar, et al. (69) report two periods of flowering in millet (Setaria italica (L) Beauv.). Two periods of flowering in Agropyron elongatum, as found by Sando, have been reported (91).

Several studies show the influence of the climatic or environmental conditions on blooming. Stephens and Quinby concluded in their studies on sorghum: "Relative humidity apparently did not influence the time of blooming." Although the hour of maximum blooming activity in sorghum varied with varieties, they state, "A relatively small proportion of flowers opened before 10:00 p.m. or after 8:00 a.m., but there were no hours in which flowers were never found opening." By reversing darkness and light, by means of artificial light and darkrooms, they were able to reverse the natural rhythm of flowering in 36 hours. Light conditions would, therefore, appear to be a most important factor in governing the time of flowering. They found, also, that reducing the temperature reduced the rate of flowering.

Fruwirth (34) agrees with Stephens and Quinby regarding the effect of humidity but not as to light. He placed potted plants of ryegrass

and orchard grass under light-tight boxes and found that, "the plants bloomed in spite of the lack of light." He concluded: "From this experiment, in which the heat was sufficient but light was lacking, the latter seems to be unnecessary for blooming." He holds temperature of much greater importance in its effect of blooming than light and moixture, but that humidity delays the opening of the anthers. Mercado (55) found that high humidity, or cloudy and rainy weather, greatly affected the flowering in sugarcane. He states that, "Opening of the flowers began about 5:30 a.m. or earlier, almost all being opened before 7:00 a.m. On rainy days few flowers opened, but the course of opening lasted the whole day." The two maxima of flowering as reported by Li, et al. (50) were at periods when the temperature was relatively high and when the relative humidity was low. Flowering in the same species was reported by Rangaswani Ayyengar, et al. (69) who found that in hot weather the periods of flowering were nearly equal in intensity, but in cold weather the second period was only half the intensity of the first. Sando (90) believes that the delay or reduction in flowering in Agropyron elongatum, caused by a cloudy sky, is due more to the lowering of temperatures than to increased humidity. Wolfe (96) states, "Temperature seems to have a marked effect on the period of blooming," and gives observations that orchard grass will not bloom below 52° F. Misonoo (57) shows that the particular time of day when blooming begins in the oat is correlated with the time the optimum temperature is reached. If this is reached earlier than

usual, blooming follows correspondingly earlier. He was able to control the flowering of the oat by varying the temperature but could not by varying humidity. Burton (23) has just recently reported his observations on the flowering of four Paspalum species. He shows that all florets blooming in any 24-hour period bloomed early in the morning but blooming was retarded on cool or moist days. Little, if any blooming was noted if plants were kept in a dark room. Blooming before dawn in P. urvillei is reported. Anther dehiscence was retarded four hours by placing panicles of P. dilatatum and P. notatum in a large cloth-covered box and creating a fine mist of water by means of an atomizer. Dehiscence of the anthers he credits as being dependent upon desiccation.

The development of the embryo in grasses has received very little attention. Studies on grass embryos have been based on observations of embryos in early stages of development, mature embryos and seedlings of mature seeds. Most of these studies were made for the purpose of gross morphological development or of determining the homologies of various portions of the embryo. Relatively little attention has been given to the important intermediate stages of development.

According to Van Tieghem (89) the earliest description of the embryo of grasses is that by Malpighi in 1687 of the embryos of Avena and Triticum. In these he recognized the structures now known as the scutellum, epiblast, and coleoptile.

Embryo and seedling morphology in various Gramineae have been studied for more than 120 years. These studies have concerned themselves chiefly with mature embryos for the purpose of determining the homologies and other principal features of the species studied in relation to those of other grasses. This is still a highly controversial subject as shown by views presented in the more recent papers by Avery (7), Howart (40), Percival (64), and Merry (56). The literature of this subject is extensive. Detailed reviews of certain phases of embryo morphology have been prepared by Van Tieghem (69), Brunn (21), Kennedy (45), and Avery (8). An extensive critical review of the literature and a summary of the various views pertaining to gross morphology and homologies has been published by McCall (54).

Early studies on the development of the embryo were based mainly on observations of whole embryos. Hofmeister (61) studied the early divisions of the zygote and proembryo of Hordeum, Avena, Triticum, and Secale by dissecting out whole embryos and mounting them in glycerine for observation. The oldest stages of which he gave figures were just beginning to show gross differentiation. He attempted to classify the arrangement of the cells according to the manner in which they divided.

Souèges (83) traced the parts of the fully developed embryo of Poa annua back to the tier of cells in the sixteen-celled proembryo. He maintained that the parts of the embryo are determined at least

as early as the sixteen-celled stage. He also indicated that there was a definite arrangement of the cells and a regular sequence of cell divisions in the development from the fertilized egg.

Randolph (68) has given a complete description of the development of a Zea embryo in which the age of the various stages was determined. He found that there was no definite arrangement of the cells nor any regular sequence of divisions in the early stages. The sectors of the proembryo in the initial stages of development were so rapidly obscured that it was impossible to determine or predict which sector or sectors gave rise to the root and shoot regions of the plumule-radicle axis. He considered temperature relations as an important factor influencing the rate of development.

Merry (56) has described the development of the embryo of Hordeum sativum from the time of fertilization to maturity of the seed. He found no definite arrangement of cells or sequence of cell divisions in the proembryo. He shows that embryos of Hordeum varying one day in age have recognizable morphological differences.

LaRue and Avery (46) show a rapid development of the embryo of Zizania aquatica, the mature embryo reaching a length of 10 mm. in 19 days. The normal embryo of this plant possesses a cotyledon which extends the entire length of the fruit and often extends around the end of the caryopsis. They show that the normal growth and development of the embryo is dependent upon association with the tissues of the plant and a normal embryo cannot be grown in artificial culture.

The only reference concerning the embryo of species of the genus Paspalum is that of Paspalum pubiflorum glabrum by Kennedy (43). The embryo of this species is described as having a large radicle, its scutellum-bundle is inserted at some distance from the plumule, there is no epiblast, and one foliage leaf is present. His description is based on the mature embryo.

Shadowsky (77) has investigated the embryo sac of Paspalum dilatatum. He describes the embryo sac as being a normal chalazal type, with 5 to 14 antipodals. A recent summary of grass embryo sac investigations is given by Stover (87).

Marchal (53) first classified Paspalum dilatatum as being a tetraploid with 20 pairs of chromosomes. Church (26) states that 10 is the basic (n) number and that this number is well established. He believes that polyploidy in this genus is responsible for lagging chromosomes, the relatively high percentage of sterile pollen, and the frequent irregularities in chromosome counts. Avdulov and Titova (6) have shown additional chromosomes in several species. Krishnaswamy (47) designates the genus Paspalum as a high polyploidy genus with 10 as the basic (n) number of chromosomes but may prove to be five in the future. He gives the karyotypes of Paspalum and Andropogon as quite similar. Later work by Saura (75) and Burton (22) give evidence that 10 is the basic (n) number of chromosomes and that species of Paspalum fall into a polyploid series ranging from 2n to 16n.

A considerable amount of investigations on artificial pollen germination, pollen storage, and pollen longevity has been reported. Only those pertinent to grasses will be reviewed.

According to a summary of work done on pollen longevity to 1926, compiled by Holman and Brubaker (39), determinations have been made of the longevity of the pollen of 231 species of plants, comprising 175 genera and 23 families. Under natural conditions the pollen of a considerable number of plants is relatively short-lived and pollination, to be effective, must take place within a very few hours after anthesis. They state that "the cereals and a number of grasses have long been recognized as having short-lived pollen." They give a range in pollen longevity of 336 days in Typha latifolia to one-half hour in Secale cereale. There is a tendency to uniformity in pollen longevity within a genus.

The short-lived characteristic of grass pollen is emphasized by Anthony and Harlan (2) who state that barley pollen exposed to the air for only 10 minutes lost its germinative properties completely. They report that a proper range of humidity must coincide with a certain range of temperature and that the water adjustment of the pollen was so delicate that it could be readily killed by blowing one's breath upon it. Their use of the artificial germination of barley pollen makes "it obvious that failures in hybridization are due much more to faulty pollen than to any lack of receptivity of the stigma." They state that the physical factors and not specific substances of the stigma were responsible for pollen germination.

Pope (67) was able to store undisturbed barley pollen for 26 days at 36° F., 19 days at 40° F., and for 14 days at 50° F. This was not pollen taken from the florets at anthesis, but pollen from culms of barley with spikes cut from the plant and placed in vessels. Weller (92), using the pollen from cut tassels of sugarcane, found that viability was lost by the third day after cutting. Mercado (55) also found that sugarcane pollen decreased rapidly in viability after two or three hours but, by preserving canestalks in solutions, he was able to bridge the differences in flowering intervals and hybridize varieties. He used fresh stigmas as a medium for testing pollen viability. Dutt (30) and Venketraman (90) give data to show that a medium containing 0.7 per cent agar and 26 per cent sucrose is best for the artificial germination of sugarcane pollen. He used such a method to test varieties and plants as parents in sugarcane breeding. This method has also been used to select parents in forest tree breeding (71).

Reyes (70) found that the best pollen germination with rice was at the time of flowering. This view is also held by Doroshenko (29) who states that grass pollen is characterized by a high water content and a rapid rate of its loss. He prolonged pollen longevity by means of low temperatures (5° to 10° C.) and relatively high humidity (60 to 80 per cent). He classed grasses as belonging to a group where sugars probably create a favorable osmotic pressure for artificial germination. A table is given containing the data for over 500 species as to the

optimal conditions for the germination of pollen in vitro, storage and longevity. All grasses reported have extremely low germination of pollen except that of maize. Tabata, et al. (88), working with 11 genera of grasses, show no germination 5 hours after pollination. They find that 15 to 45 per cent sugar in dilute agar solution proved best for the germination of grass pollen. They also give a low per cent germination for grass pollen.

Bair and Loomis (9) have obtained as much as 90 per cent germination of corn pollen on a nutrient medium containing 0.7 per cent agar and 15 per cent sucrose. They state that the balance between the nutrient medium and the cytoplasm should be slightly on the hypotonic side so that the pollen grain may absorb water for tube growth, but not rapidly enough to cause bursting. Best results were obtained when the grains were two-thirds imbedded and one-third exposed to the air and at a humidity of 90 per cent.

Other uses have been made of the artificial germination of pollen. Sisa (79) believes that pollen germination can be used successfully for the distinction of the species of the genus Brassica. Winkler (94) found that with an increase in the sugar content of mature grape flowers there was a marked increase in the germinability of pollen and setting of berries. MacDaniels and Furr (52) tested the effect of sprays and dusts upon pollen by artificial germination and subsequent fruit set in the apple.

There is some controversy as to the ability to germinate in culture in relation to ability to affect fertilization. Knowlton (45) states that pollen may be able to germinate and yet be incapable of functioning in fertilization. Nohara (60) found that pollen can be used for fertilization as long as it is able to germinate. Romer (39), on the other hand, believes that pollen which has lost its power to germinate artificially is still capable of fertilizing ovules. Pfeiffer (65) used both stigmas and artificial media in testing the longevity of stored Lilium pollen. Passecker (63) gives data from two varieties of apples, two of pears, and one of plum which were self- and cross-pollinated, showing that there is a positive correlation between artificial germination and the ability of the pollen to effect fruit setting. Kesseler (44), artificially germinating the pollen of potato, states that the ability of the pollen to germinate in vitro is indicative of the capacity to grow in vivo to the ovule.

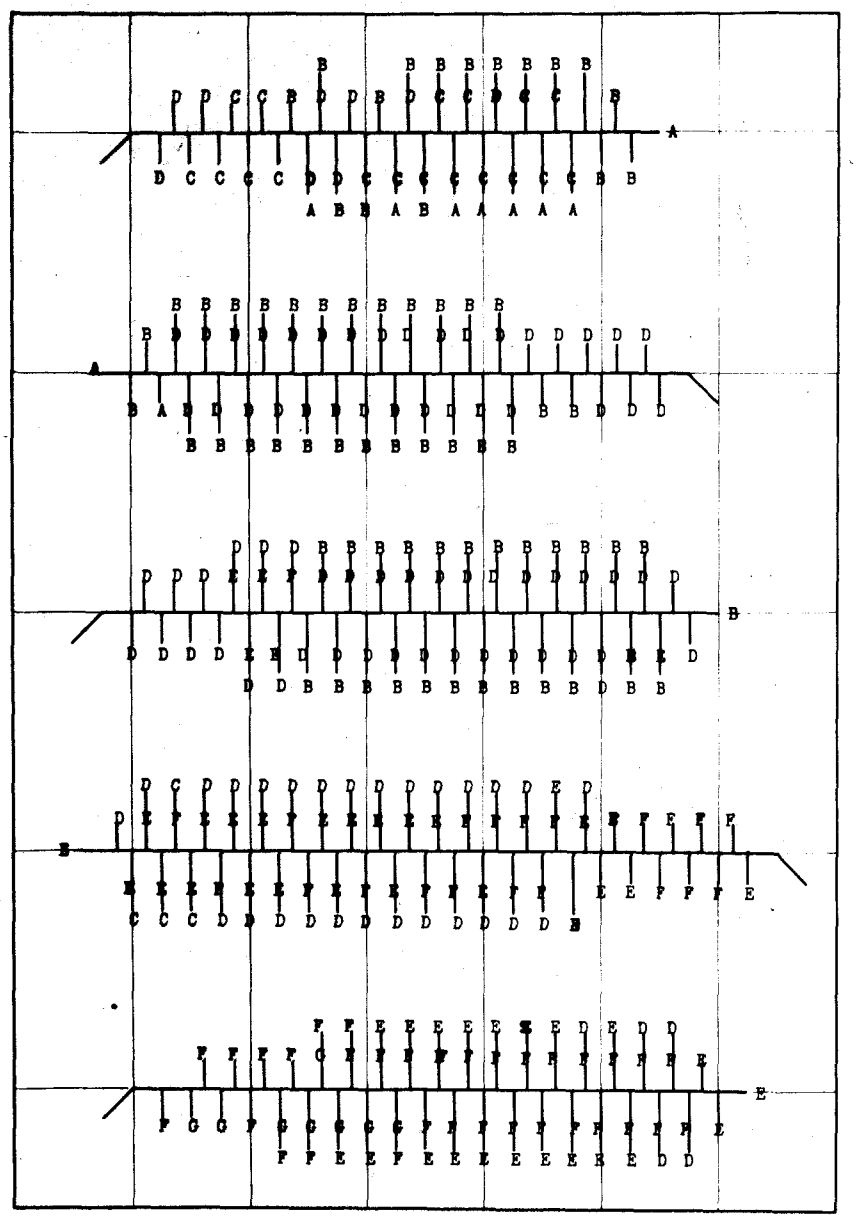
Sartoris (73) recently reported that the water relations of grass pollen are very delicate and concentration must be exact before germination takes place. He states that "The ability of sugarcane pollen stored from four to seven days to fertilize shows that germination on artificial medium is a test of the ability of sugarcane pollen to function normally." He finds that germination obtained one day may not be repeated with the same conditions of concentration, temperature, pH, and humidity. He gives widely varying percentages of germination of sugarcane pollen.

Poole (66), working with hybrids of Crepis species, has found that in hybrid plants differences in pollen production vary with the genetic constitution of the hybrids, the more fertile genetic constitutions possessing higher proportions of good pollen than the less fertile constitutions. He made pollen studies among hybrids which were being selected for fertility. This permitted the raising of larger generations by reducing the required cytological examinations. Milford (59) found no correlation between per cent "good" pollen and fertility in chrysanthemums but states, "The lack of any correlation is to be expected, however, since this group of plants seems to be a mixture of rather complex hybrids." He noted variations in the size of pollen grains from the same plant.

MATERIALS AND METHODS

Detailed hourly counts of flower opening were made on the racemose inflorescences of Paspalum dilatatum, P. malacophyllum, and P. urvillei. The day and hour of flowering were recorded on diagrams of each inflorescence as shown by Figure 7. Observations just before dawn were made with a small flashlight which was upon the heads only long enough to secure the necessary counts. The flower was considered as having bloomed as soon as the anthers had become extended far enough to allow quick and easy removal. Temperatures and humidities were recorded by means of a hygrothermograph.

Plants of the three species were grown in 5-gallon earthenware pots filled with screened Bell silty clay loam soil. This soil is typical of the "Prairie" section of Mississippi. Two treatments were used to test the effect of soil moisture upon flowering. One treatment consisted of holding the soil just below its total water holding capacity, and a comparative treatment of 15 per cent of the water holding capacity. Fifteen per cent of the water holding capacity was chosen because this is the status of the moisture in this soil during many dry periods of the summer and is below the optimum water holding capacity of this soil for plant growth. These levels of soil moisture were kept constant by weighing the pot and plant at 48-hour intervals. Flowering counts were recorded by means of diagrams of



ORDER OF FLOWERING IN A PANICLE

Figure 7.

each head to be counted.

Potted plants of each species, after the second day of flowering under natural conditions of temperature, light, and humidity, were placed in a dark-room at 8:00 a.m. Artificial light was given during the afternoon (6:00 p.m.) of the same day. The plants had received 10 hours of darkness. Thereafter the plants were kept in light 12 hours of the natural night (6:00 p.m. - 6:00 a.m.) and in darkness 12 hours of the natural day (6:00 a.m. - 6:00 p.m.). Under these conditions the normal relations of light and darkness to humidity and temperature were reversed as shown by the hygrothermograph readings given in Table 8.

Potted plants of each species were allowed to complete their second day of flowering. They were then placed in a control room where the temperature was held constant (within a four degree range) and the normal relations of light and humidity were reversed. Daily and hourly counts were recorded on diagrams of the inflorescences to be counted.

Plants in full flowering were placed in the control room at 6:00 p.m. The temperature had been adjusted to that desired. The humidity was held at 70 per cent relative humidity. Lighting began at 6:00 a.m. The plants had 12 hours of light during the natural day and 12 hours of darkness during the natural night at the desired temperature and 70 per cent relative humidity. The number of florets blooming in 10 inflorescences was counted each day for five days. Different plants

were placed in the room each day. Temperatures for each five-day period were reduced in intervals of five degrees, until the temperature at which flowering would not take place was determined. The time required for the process of flowering in the individual floret was taken on 20 florets of each species, for five days at each temperature interval. This gave the time required for flowering in 100 florets at each temperature.

Material for embryological studies was obtained from potted plants of strain 56-17 growing in a shaded greenhouse. Florets that had bloomed were removed from the inflorescences the third day of blooming. Florets blooming before 5:30 a.m. of the fourth day were removed before that time on the fourth day. Normal flowering was completed the fourth day. Racemes were rubbed every half-hour to insure pollination. All spikelets on the inflorescence that had not bloomed on the fourth day were removed after flowering had been completed on that day. Inflorescences were then labelled with dated tags. This procedure was continued intermittently from June 15 to September 1.

Samples were collected at desired intervals. It was necessary to open each spikelet and remove the growing seed or infertile ovule with fine-pointed forceps because of the indurate nature of the lemma and palea. Counts of infertile ovules showed that 72 per cent were dried and thus not fertilized.

The material was killed in Craf I and Allen-Bouin (74) fluids. Suction was applied to material younger than six days to give total

immersion. Material older than 8 days was embedded in paraffin through a butyl-alcohol series. Paraffin sections 10μ to 15μ in thickness were stained with Delafield's haematoxylin, and also in safranin-fast green.

Root tips were obtained from potted plants of each species and their hybrids. Collections were made between 9:00 a.m. and 11:00 a.m. These were fixed in Craf III for one day and embedded in paraffin. Sections were cut 8μ in thickness and stained in gentian-violet-iodine and in iron-hematoxylin.

Pollen from dehiscing anthers was dusted on clean microscopic slides. These were then prepared according to the method described by Wodehouse (95). Pollen size was determined by measuring the plump or rounded grains in four microscopic fields per slide with an eyepiece micrometer at low-power. Shrunken or shriveled grains were designated as bad pollen and were counted but not measured. Five slides were used for each species. Counts and measurements were made intermittently throughout the growing season.

The various media for artificial pollen germination trials were made up the day before each test. Solutions were sterilized and adjusted to the desired pH and capped for use the following day. Agar was likewise sterilized and the pH adjusted. Each agar treatment was placed in its appropriate place in a 9-hole spot plate by means of an eye-dropper. Enough was added so that when it had solidified the hole would be a little more than full. Another spot-plate was inverted over the filled plate until morning. Solutions were placed

just before anthesis. Clonal plants of a good seed producer (56-17) and a low seed producer (11-48) were used. Inflorescences with dehiscing anthers were shaken over the plates. The plates were then placed in desiccators and the whole placed in the oven at the desired temperature. Control of relative humidity was attained with sulfuric acid and water in the proportions as listed by Loomis and Shull (51) to obtain 85 per cent relative humidity. Plates containing solutions were not placed under varying humidities. Preliminary studies included type of sugar, gelatin-agar mixtures, and relative humidity carried on in the same manner. Germination counts were made on four low-power microscopic fields within each treatment approximately 2 hours after pollen placement.

Measurements of spikelets were made as a matter of interest by means of a wide field, low-power magnifying glass. Spikelets in each row of the four-rowed racemes of an inflorescence were measured throughout the seasons.

EXPERIMENTAL RESULTS

Flowering

The results of detailed hourly counts of flower opening, under average summer conditions, are given in Tables 1, 2 and 3 and shown graphically by Figure 8. It will be noted that Paspalum urvillei begins to flower vigorously between 2:00 a.m. and 3:00 a.m., reaching its maximum at 5:00 a.m., at which time the rate begins to decrease rapidly. Paspalum malacophyllum begins to flower during the hour before dawn, and reaches its maximum within an hour afterwards. Paspalum dilatatum begins meager flowering between 4:00 a.m. and 5:00 a.m., reaching its maximum between 6:00 a.m. and 7:00 a.m. This is two hours after the maximum of P. urvillei and three hours after the maximum of P. malacophyllum, indicating that the latter species is more sensitive to temperature than to light. The maximum of flowering in P. dilatatum occurring three hours after dawn would indicate that this species needs some period of light before intensive flowering takes place. The average temperature over this period of flowering was from 70° F. at 3:00 a.m. to 81° F. at 9:00 a.m. (Table 4). Relative humidity ranged from 81 per cent at 3:00 a.m. to 61 per cent at 9:00 a.m. The small range in both temperature and humidity indicates that light was the most important factor within these ranges.

Table 1. The Average Number of Flowers Blooming Each Hour in 15 Inflorescences of Paspalum Urvillei During the Blooming Period

Day of blooming	Number of flowers opening at indicated hours. A.M. 2-3	3-4	4-5	5-6	6-7	7-8	8-9	Total by days
1			2					2
2		1	13	3	2			19
3	3	8	2	5				18
4	39	10	1	21				71
5	103	17	4	12	2			138
6	88	38	44	55	29	17		271
7	73	44	45	73	23	10		268
8		164	154	30	39	35	3	425
9		61	98	3	8	2		172
10		13	80	1	3			97
11		7	56	4				67
12		7	35	1				43
13		15	1					16
Total by hours	306	385	535	208	106	64	3	1607

Table 2. The Average Number of Flowers Blooming Each Hour in 15 Inflorescences of Paspalum dilatatum During the Blooming Period

Day of blooming	Number of flowers opening at indicated hours. A.M.							
	2-3	3-4	4-5	5-6	6-7	7-8	8-9	Total by days
1			8		41		4	53
2			6	33	63		1	102
3	1	2	22	29	41	20	1	116
4	3	10	19	34	39	47		152
5	1	6	14	43	33	21	1	119
6		1	3	32	31	5		73
7			4	7	11		2	24
8			1	3	5			9
9			1	1	2		1	5
10			1	1	2			1
Total by hours	5	19	78	182	267	93	10	654

Table 3. The Average Number of Flowers Blooming Each Hour in 15 Inflorescences of *Paspalum malacophyllum* During the Blooming Period

Day of bloom-ing	Number of flowers opening at indicated hours. A.M.							Total by days
	2-3	3-4	4-5	5-6	6-7	7-8	8-9	
1			1	8	4	1		14
2		42	33	26	26	3		130
3	7	114	32	16	16	2		187
4	29	134	26	14	5	1		209
5	2	143	55	34	4			238
6		63	31	18	16	4	7	139
7			1	3	11	11		26
8			23	37	14	9		83
9				5	2	4		11
10		5	13	8	6	3		33
11		4	9	5	2	1		21
Total by hours	38	503	224	174	106	39	7	1091

Table 4. Temperature and Humidity Readings at Each Hour During the Blooming Period of the Paspalum sp. in Tables 1, 2 and 3

Day of bloom-ing		Readings at hours indicated. A.M.							
		2	3	4	5	6	7	8	9
1	T	74	74	74	76	78	80	82	90
	H	80	80	80	80	80	76	70	64
2	T	72	72	73	73	74	76	80	82
	H	80	80	78	75	70	60	56	46
3	T	72	72	72	72	74	78	82	90
	H	80	80	80	80	70	60	50	44
4	T	74	73	72	72	76	80	84	88
	H	78	78	78	78	66	60	56	50
5	T	71	71	71	71	74	80	88	92
	H	78	78	78	76	72	58	50	44
6	T	68	68	68	69	70	74	78	80
	H	81	81	81	81	80	66	62	60
7	T	70	69	69	69	70	71	73	78
	H	80	80	80	80	80	76	72	62
8	T	70	70	70	70	70	72	74	78
	H	82	82	80	80	80	76	72	70
9	T	70	70	70	70	70	71	71	71
	H	84	84	84	84	84	86	86	86
10	T	70	70	70	71	72	76	78	86
	H	84	84	84	84	80	74	64	56
11	T	71	71	71	71	72	74	76	84
	H	80	80	80	80	78	74	70	50
12	T	70	69	69	70	72	78	84	88
	H	82	82	82	80	70	62	50	42
13	T	72	72	72	72	72	78	84	90
	H	80	80	80	80	70	58	46	40

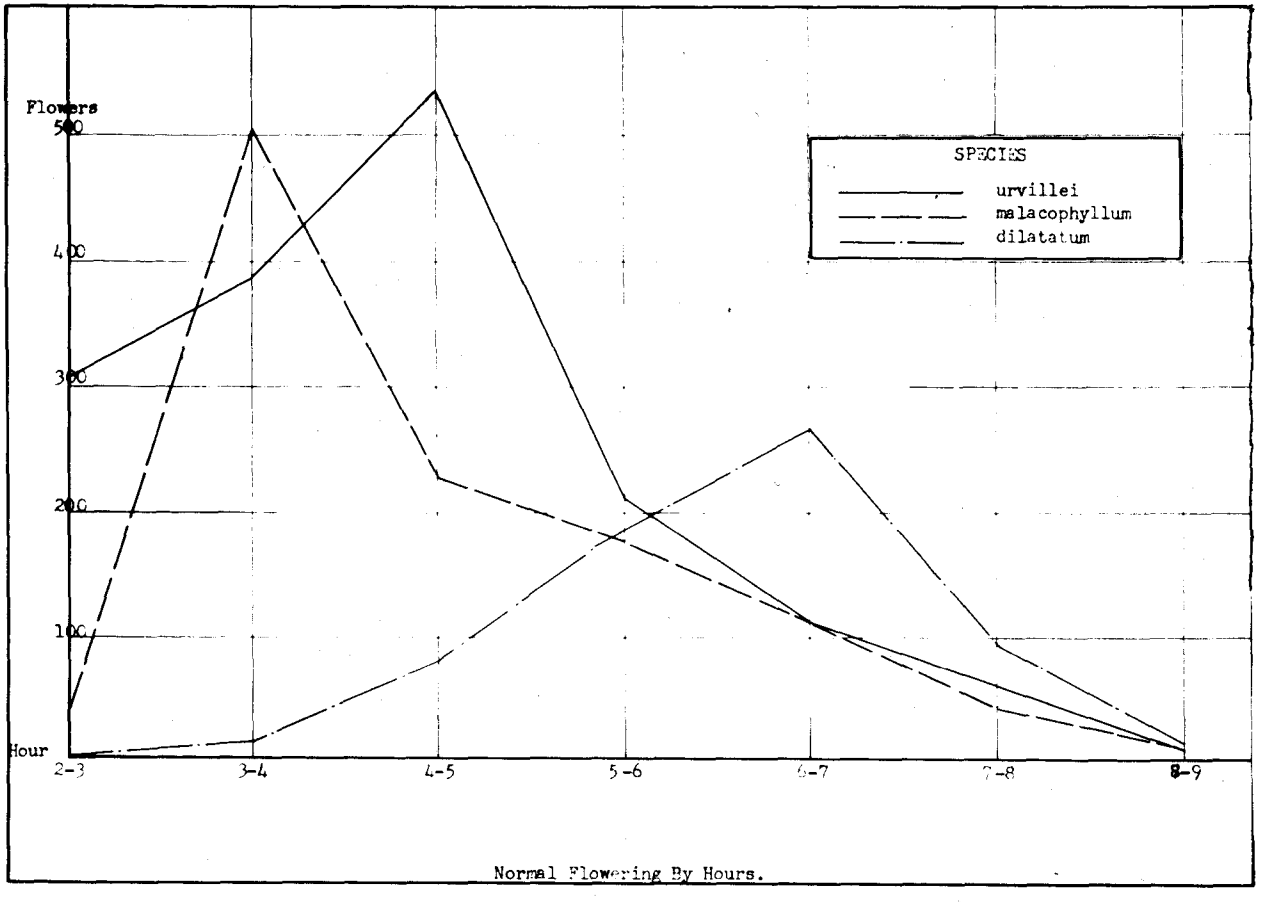

Flowers
500
400
300
200
100
Hour
2-3
3-4
4-5
5-6
6-7
7-8
8-9
SPECIES
urvillei
malacophyllum
dilatatum
Normal Flowering By Hours.

Figure 8.

It should be kept in mind that the glumes of many of the flowers may have been fully spread at the hour of counting but that the anthers were not fully pendent and therefore the floret was not counted as having opened. Flowering may have occurred almost an hour previous in many of the florets. This was approximately the same for all species but it indicates that the amount of flowering may be concentrated within a shorter time in species that are highly influenced by light. No secondary period of flowering has been observed in any of the three species studied.

The time required for all flowers of the spike-like racemes to open varies somewhat with the size of the inflorescence but averages 13 days for P. urvillei, 11 days for P. malacophyllum and 9 days for P. dilatatum.

The rate of flowering by days is shown in Figure 9. The peak of flowering is reached the fourth day in P. dilatatum, the fifth day in P. malacophyllum, and the eighth day in P. urvillei. The average total number of flowers per inflorescence was 654 in P. dilatatum, 1091 in P. malacophyllum, and 1607 in P. urvillei. The averages shown graphically in Figure 4, are within the temperature and humidity ranges which usually exist during the summer months.

The three species have the same order of flowering. Flowering proceeds from the top raceme down the racemose inflorescence, and from the end-most spikelet back toward the base of the raceme. The florets

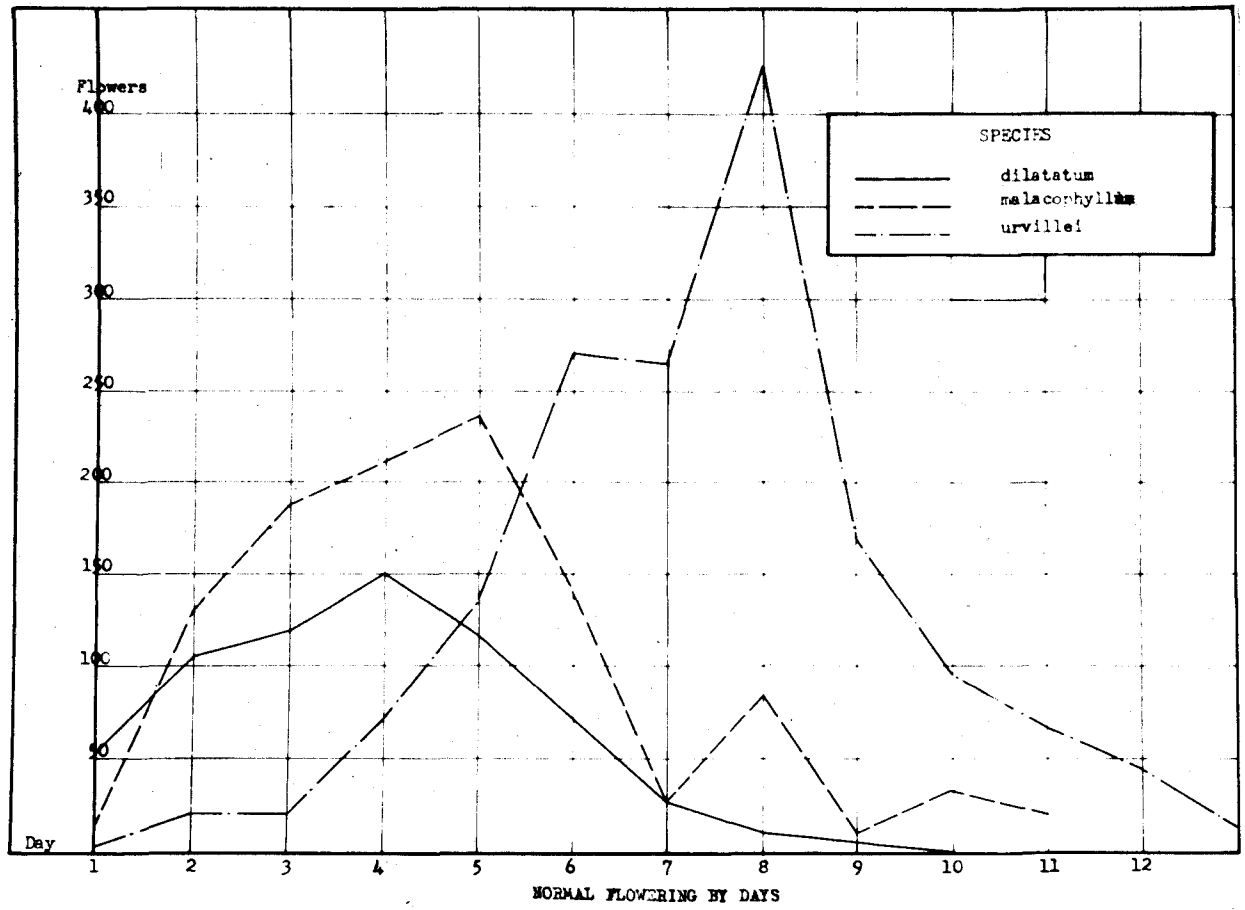

Flowers
400
350
300
250
200
150
100
50
Day
1
2
3
4
5
6
7
8
9
10
11
12
SPECIES
dilatatum
malaconhyllum
urvillei
NORMAL FLOWERING BY DAYS

Figure 9.

of the paired spikelet usually flower on alternate days. This is not a fast rule as some instances of both spikelets flowering the same day have been noted. Beginning at the end of the raceme and proceeding downward, the outside florets open and are followed by opening of the inside ones. The outer spikelet of the paired spikelets always flowers first. The order of the flowering is shown by the diagramatic drawing (Figure 7) of the five racemes of a P. dilatatum inflorescence. This is characteristic of the order in the three species.

Figure 10 shows the flowering of each species grown on soil of low moisture content, as compared to the flowering on soil kept at its total water holding capacity. These data are recorded in Tables 5, 6 and 7. The low moisture content treatment is referred to as "normal" and the total water holding capacity treatment is "irrigated". Soil moisture had little effect upon the rate of flowering of the three species. High soil moisture lengthened the flowering period by one day. The number of florets opening on this extra day did not exceed 25 florets per inflorescence of any species. Plants of each species growing on soil of high moisture content produced a larger inflorescence than those growing on soil of low moisture content. This accounts for the greater numbers of florets on the "irrigated" plants.

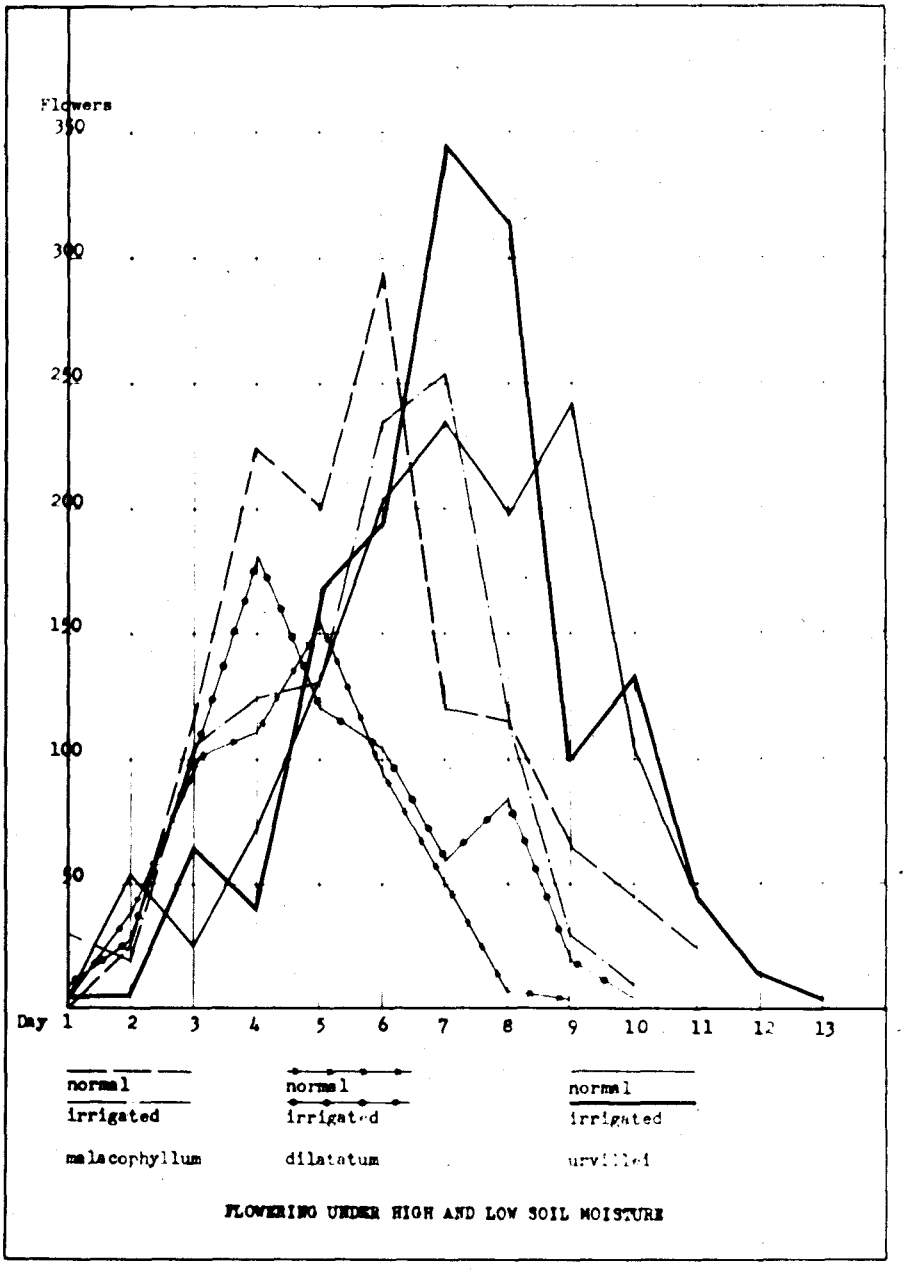

Flowers
350
300
250
200
150
100
50
Day 1 2 3 4 5 6 7 8 9 10 11 12 13
normal
irrigated
malacophyllum
normal
irrigated
dilatatum
normal
irrigated
urvillei
FLOWERING UNDER HIGH AND LOW SOIL MOISTURE

Figure 10.

Table 5. Flowering of Paspalum urvillei on Soil at High and Low Moisture Levels. Average of 10 Inflorescences.

Day of bloom-ing	Number of flowers opening at indicated hours. A.M. 2-3	3-4	4-5	5-6	6-7	7-8	8-9	Total by days
Bell silty clay soil at 15 per cent water holding capacity								
1		1	4	2				7
2		7	23	16	5	2		53
3	2	3	9	7	3			24
4	26	11	15	12	6	1	1	72
5	19	16	47	32	9	7		130
6	37	49	58	43	13	2		202
7	1	23	96	81	15	18	2	236
8	18	37	49	72	11	9	1	197
9	22	23	127	48	19	7		246
10	9	33	51	9	7			109
11		18	22	4				44
12		12	3					15
Total by hours	134	233	504	326	88	46	4	1335
Bell silty clay soil at total water holding capacity								
1			3	1				4
2		2	1	5	1			9
3	9	3	18	15	9	7	1	65
4		13	15	7	3	3		41
5	4	29	101	18	9	5	1	167
6	29	31	75	41	11	4	2	193
7	15	76	156	64	24	8	1	344
8	32	51	143	60	14	14	2	316
9		29	36	28	17	11		101
10		19	64	33	11	9		136
11		20	18	6	2			46
12		1	12	3				16
13			2					2
Total by hours	89	277	644	281	101	61	7	1460

Table 6. Flowering of Paspalum dilatatum on Soil at High and Low Moisture Levels. Average of 10 Inflorescences

Day of blooming	Number of flowers opening at indicated intervals						Total by days
	2-3	3-4	4-5	5-6	6-7	7-8	8-9	
Bell silty clay soil at 12 per cent water holding capacity								
1			1	2	1			4
2		1	3	10	21	2		37
3		3	4	9	71	11	2	100
4		1	11	31	57	9	1	110
5			15	46	77	19		157
6			6	29	43	15	3	97
7			3	19	21	8		51
8			1	3	2	1		7
9				2	1	1		4
Total by hours		6	44	151	294	66	6	567
Bell silty clay at total water holding capacity								
1			1	3	6			10
2			3	9	9	4		25
3			17	26	49	9	1	102
4		3	20	53	92	16		184
5		1	14	37	56	13	2	123
6			18	32	46	9	3	108
7			3	19	28	11		61
8		1	8	20	37	16	2	84
9			1	3	16	1		21
10				1	2			3
Total by hours		5	85	203	341	79	8	721

Table 7. Flowering of Paspalum malacophyllum on Soil at High and Low Moisture Levels. Average of 10 Inflorescences.

Day of blooming	Number of flowers opening at indicated hours. A.M. 2-3	3-4	4-5	5-6	6-7	7-8	8-9	Total by days
Bell silty clay soil at 12 per cent water holding capacity								
1		18	14					32
2	5	12	3	1				21
3	7	67	18	11	4			107
4	12	60	22	21	5	2		122
5	2	82	19	12	7	5	1	128
6	3	108	62	22	12	9	2	238
7	9	139	64	29	17			258
8		79	18	8	5	3		113
9		13	12	4	1			30
10		11	2	1				14
Total by hours	38	589	254	109	51	19	3	1063
Bell silty clay soil at total water holding capacity								
1		1	2					3
2	7	11	5					23
3	11	81	13	8	3	1		117
4	26	110	45	29	13	3		226
5	14	103	24	38	16	2	1	198
6	11	136	63	41	26	13		290
7		72	29	15	4	1		121
8	3	62	32	11	8			116
9	1	38	19	6				64
10		30	9	4	1			44
11	4	16	2	1				23
Total by hours	77	660	243	153	71	20	1	1225

The hourly rate and peak of flowering is not changed by these wide differences in soil moisture either between or within species. This may be seen from Figure 11. A comparison of Figures 8 and 11 will show that the three species maintain their relative times of maximum flowering under high or low soil moisture conditions.

The normal alternationsof light and darkness were reversed by the use of a dark room and artificial light. The period of artificial darkness from the cessation of flowering (8:00 a.m.) to the time of artificial lighting (6:00 p.m. of the same day) totalled 10 hours. The data in Table 9 show that all species responded to the change by flowering within an hour after being placed in light.

Figure 12 shows that when the normal relations of light and dark are reversed, P. dilatatum keeps its regularity in flowering and that some period of light is needed before its maximum of flowering is reached. The order is the same under the normal relations of light and darkness to temperature and humidity.

Paspalum urvillei (Figure 13) showed much the same tendency as P. dilatatum. Many florets of the former had bloomed during the period of darkness but the maximum of blooming was reached one hour after the light was supplied and flowering decreased rapidly thereafter. P. malacophyllum (Figure 14) also reaches its maximum one hour after artificial lighting but had flowered considerably before lighting. The day following reversal, this species resumed its regularity by flowering just before lighting. The time required for opening and closing of the florets was greatly reduced by increased temperature.

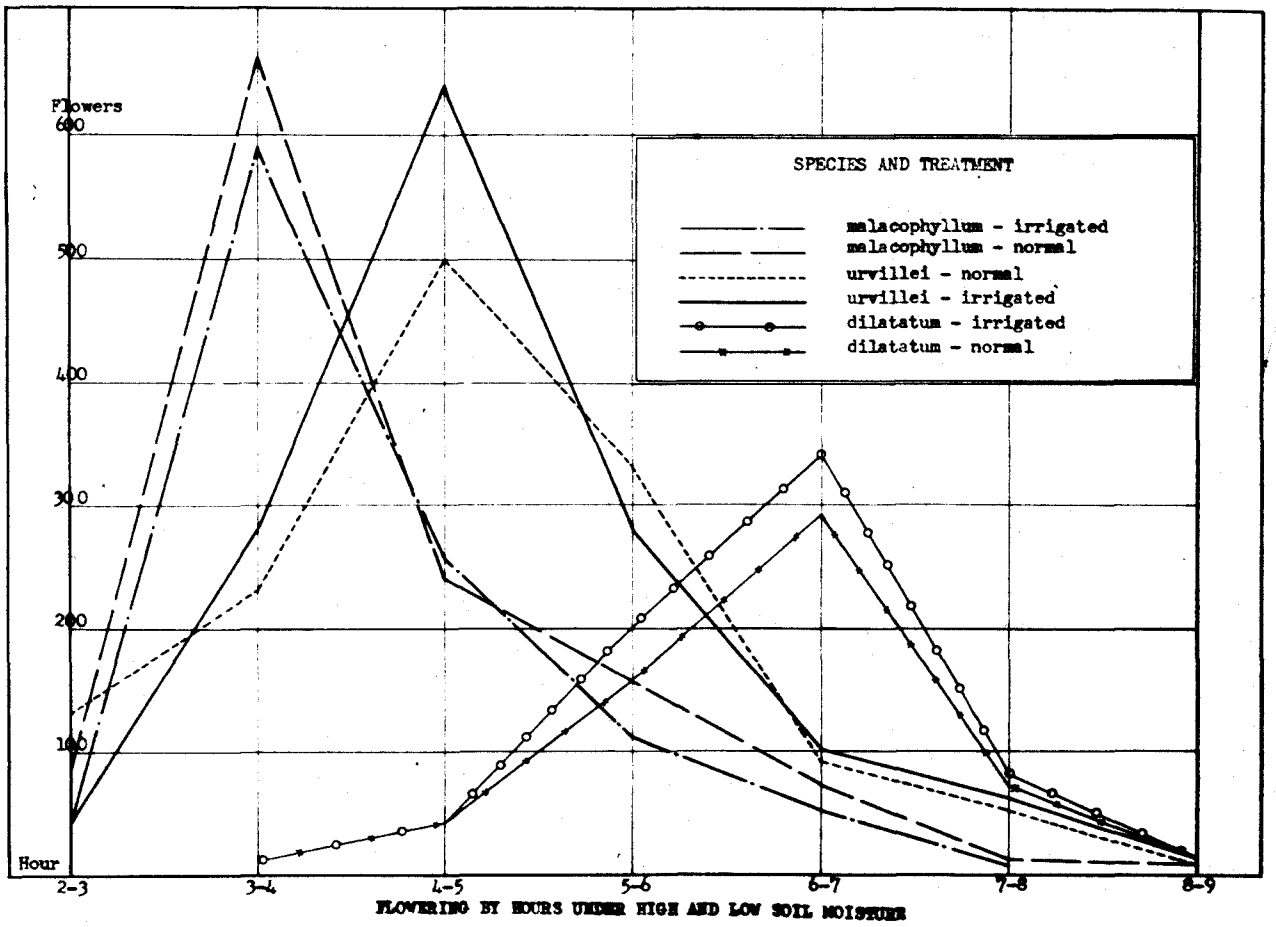

Flowers
600
500
400
300
200
100
Hour
2-3
3-4
4-5
5-6
6-7
7-8
8-9
SPECIES AND TREATMENT
malacophyllum - irrigated
malacophyllum - normal
urvillei - normal
urvillei - irrigated
dilatatum - irrigated
dilatatum - normal
FLOWERING BY HOURS UNDER HIGH AND LOW SOIL MOISTURE

Figure 11.

Table 8. Temperature and Humidity Readings at Each Hour of the Blooming Period of Paspalum sp. Before and After Changing the Natural Relation of Light and Darkness to Temperature and Humidity.

Day of blooming		Readings at hours indicated					
			4-5	5-6	6-7	7-8	8-9
A.M.	1	T	70	74	76	80	88
		H	98	88	82	62	54
A.M.	2	T	72	74	76	82	89
		H	96	80	68	58	50
P.M.	2½	T	88	86	84	84	82
		H	54	62	66	74	80
P.M.	3½	T	90	87	82	80	80
		H	50	60	72	82	84
P.M.	4½	T	86	83	81	80	78
		H	54	62	63	70	79
P.M.	5½	T	76	76	76	75	74
		H	66	72	78	80	80
P.M.	6½	T	86	80	76	74	74
		H	62	72	80	86	86
P.M.	7½	T	88	84	82	80	78
		H	58	64	74	80	80
P.M.	8½	T	78	74	72	71	70
		H	84	86	88	87	90

Table 9. The Average Number of Flowers Blooming in 10 Inflorescences of Paspalum sp. Before and After Changing the Natural Relation of Light and Darkness to Temperature and Humidity. Average of 10 Inflorescences.

Species	Day of blooming	Number of flowers opening at indicated hours						
		3-4	4-5	5-6	6-7	7-8	8-9	9-10
P. dilatatum	1		4	11	23	3		
	2		5	12	42	18		
	2½				11	36	6	
	3½			2	4	30	16	5
	4½			2	5	39	7	4
	5½			1	19	37	11	3
	6½				28	19	2	
	7½					1	3	
	8½					1		
P. urvillei	1	9	38	5	1			
	2	4	10	15	4			
	2½				16	46	7	
	3½			91	123	8	3	
	4½			33	74	11		
	5½				59	23		
	6½			58	68	21	9	
	7½			9	38	6		
	8½			4	7	2		
P. malacophyllum	1	2	4	17	6			
	2		22	19	13	7		
	2½				45	5	3	
	3½			96	1			
	4½			82	74	15		
	5½			25	21	6		
	6½			9	9			
	7½			6	8	1		
	8½				1			

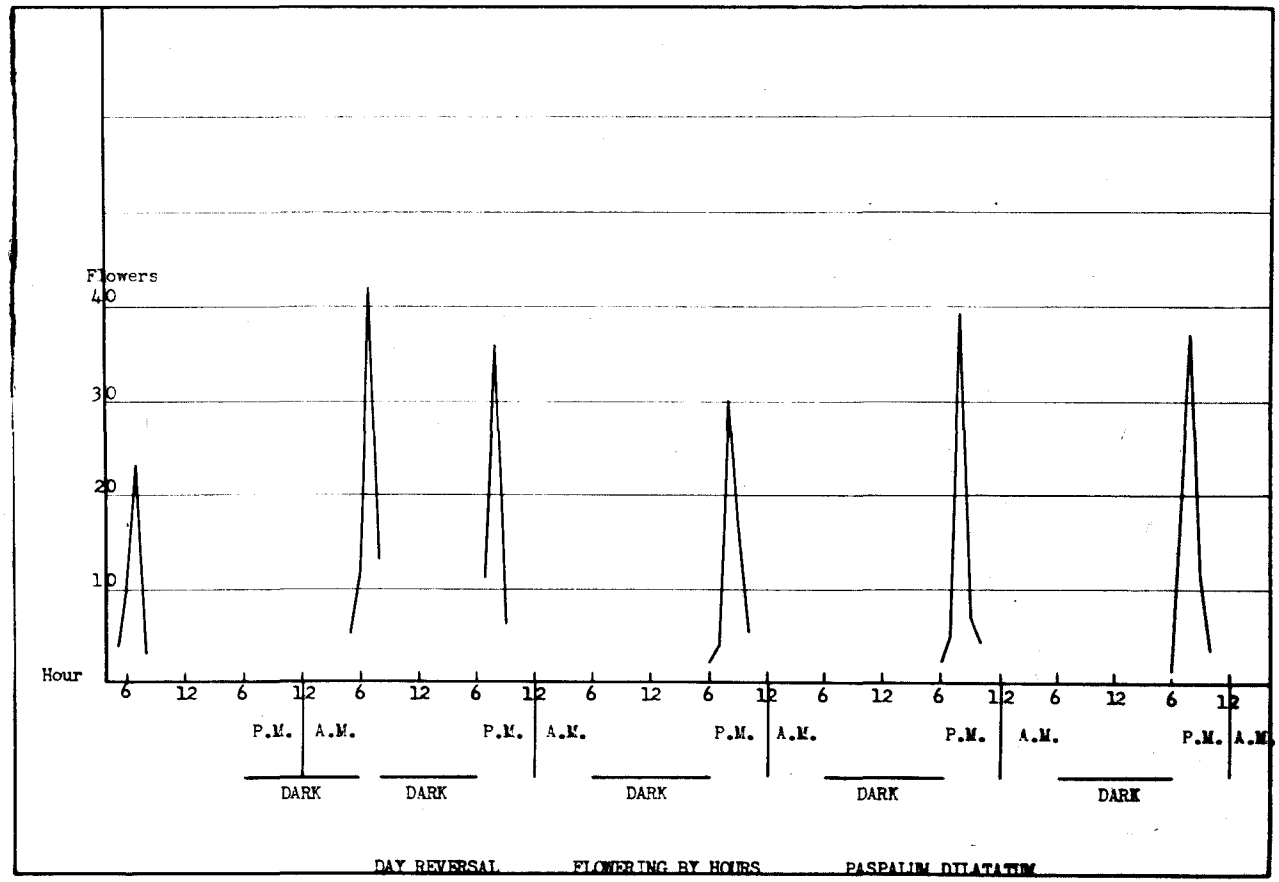

Flowers
40
30
20
10
Hour
6 12 6 12 6 12 6 12 6 12 6 12 6 12 6 12 6 12 6 12 6 12
P.M. A.M. P.M. A.M. P.M. A.M. P.M. A.M. P.M. A.M.
DARK DARK DARK DARK DARK
DAY REVERSAL FLOWERING BY HOURS PASPALUM DILATATUM

Figure 12.

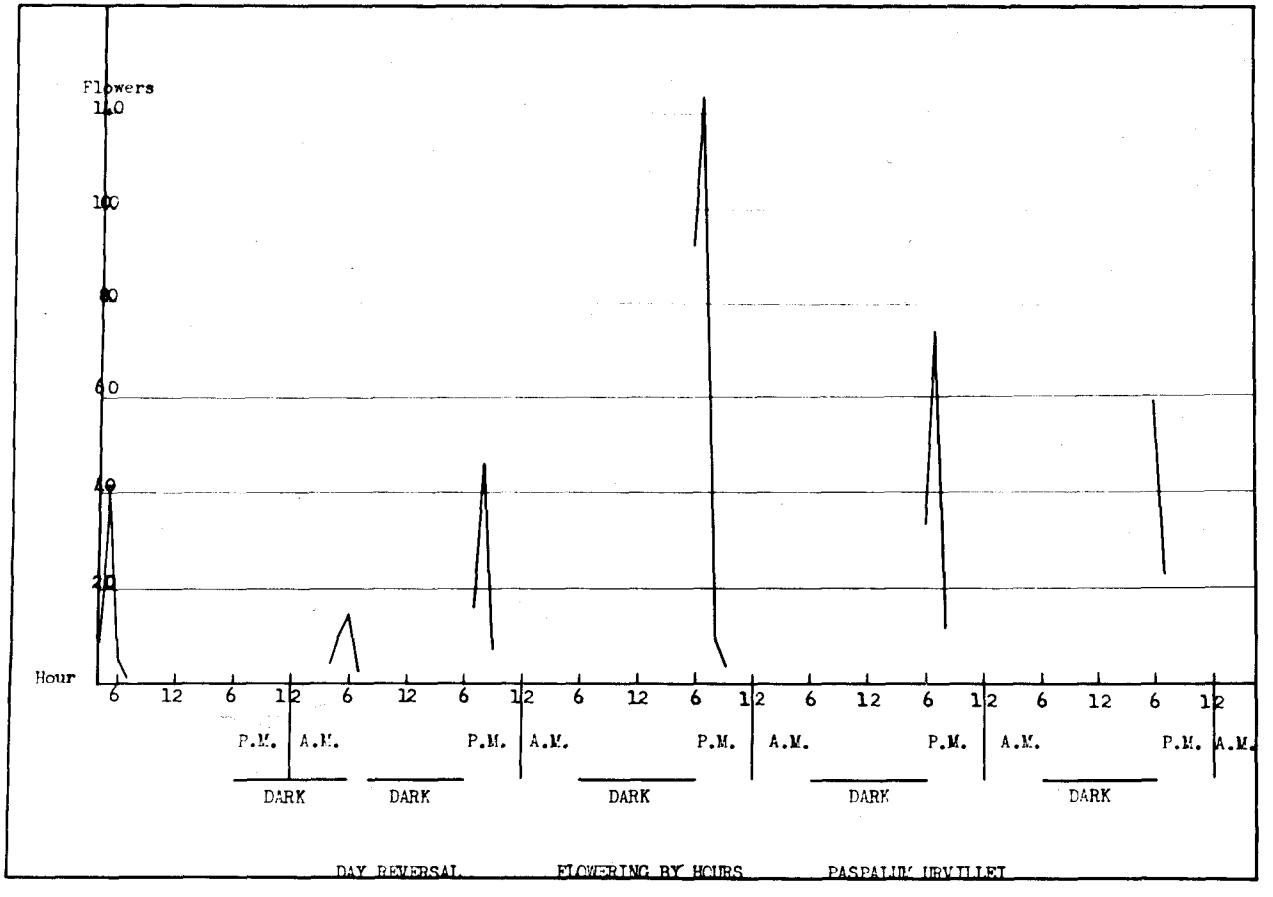

Flowers
120
100
80
60
40
20
Hour
6 12 6 12 6 12 6 12 6 12 6 12 6 12 6 12 6 12 6 12 6 12
P.M. A.M. P.M. A.M. P.M. A.M. P.M. A.M. P.M. A.M.
DARK DARK DARK DARK DARK
DAY REVERSAL FLOWERING BY HOURS PASPALUM URVILLEI

Figure 13.

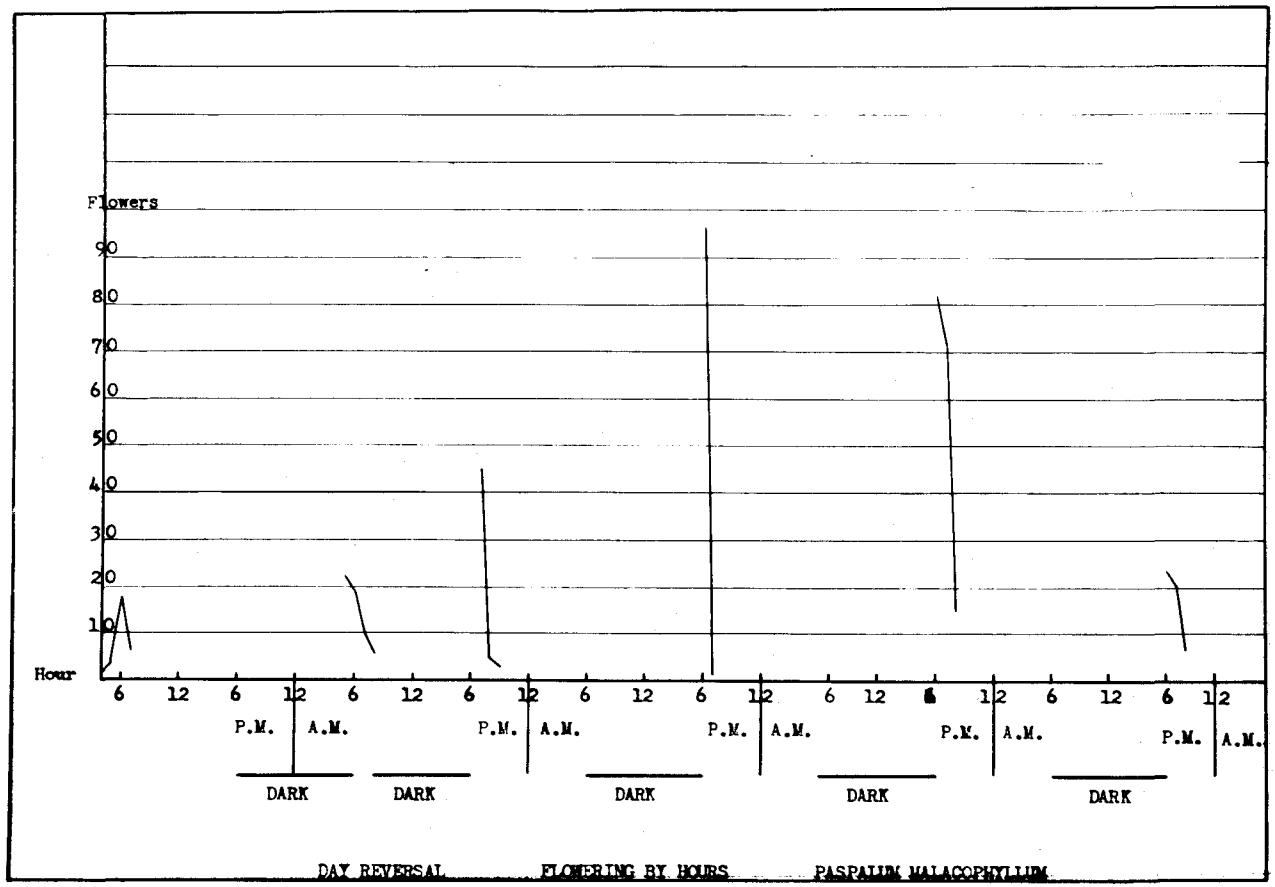

Flowers
90
80
70
60
50
40
30
20
10
Hour
6 12 6 12 6 12 6 12 6 12 6 12 6 12 6 12 6 12 6 12 6 12 6 12 6 12
P.M. A.M. P.M. A.M. P.M. A.M. P.M. A.M. P.M. A.M.
DARK DARK DARK DARK DARK
DAY REVERSAL FLOWERING BY HOURS PASPALUM MALACOPHYLLUM

Figure 14.

The hourly maxima of flowering were in the same relative position (Figure 15) as when under the normal relations of light and darkness to humidity and temperature. Lowering the temperature and raising the humidity, within this range, did not induce the three species to begin flowering or to reach their maxima at the same time. No attempt was made to determine the minimum duration of darkness necessary to initiate flowering after lighting, but plants kept in continuous light failed to bloom. Plants kept in a darkroom during the natural night and observed the next day at 9:00 p.m. had bloomed, but P. dilatatum only sparsely. Reversing the normal relations of light and darkness to humidity and temperature reverses the normal flowering thythm. It also halves the time required for blooming in the individual floret, the entire process requiring only 30 minutes. At this rate of blooming the anthers have dehisced before becoming pendent. Within the temperature and humidity range used (Table 8), P. malacophyllum and P. urvillei are more affected by either temperature or humidity than is P. dilatatum (Figures 12, 13 and 14).

Table 10 gives the average number of florets blooming before and after changing the natural conditions of light and darkness to humidity (Table 11), with constant temperature after the second day. The species bloomed in a normal manner under these conditions. The hourly maximum of blooming for each of the three species was in the same relative position as when under normal relations of light and darkness to humidity and temperature. The comparison of the time of flowering under con-

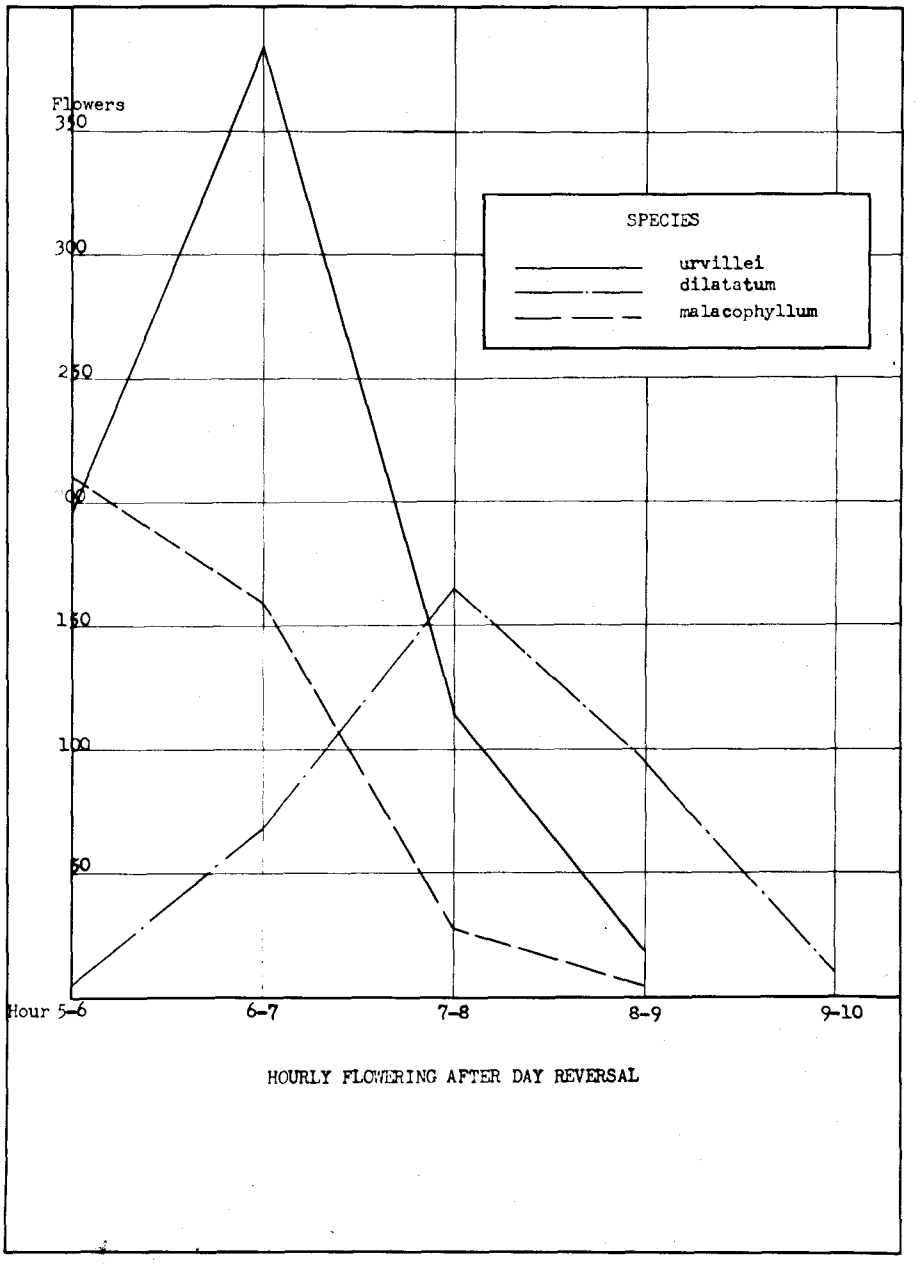

Flowers
350
300
250
200
150
100
50
SPECIES
urvillei
dilatatum
malacophyllum
Hour 5-6
6-7
7-8
8-9
9-10
HOURLY FLOWERING AFTER DAY REVERSAL

Figure 15.

Table 10. The Average Number of Flowers Blooming in 10 Inflorescences of Paspalum sp. with Constant Temperature and Where the Relative Humidity Was Reversed in Its Normal Relation to Light

Species	Day of blooming	Number of flowers opening at indicated hours						
		3-4	4-5	5-6	6-7	7-8	8-9	9-10
P. dilatatum	1			12	11	1		
	2		15	32	58	8		
	2½				9	44	7	1
	3½			2	13	55	13	3
	4½			1	16	49	9	4
	5½				17	30	1	1
	6½				1	6	2	
	7½					5	1	
	8½						1	
P. urvillei	1	8	15	41				
	2	8	7	11	8			
	2½				35	7	5	
	3½			27	36	5	2	
	4½			17	65	19	3	
	5½			23	92	72	0	
	6½				60	20	2	
	7½			7	31	15		
	8½			1	3			
P. malacophyllum	1	4	4	17	9			
	2	3	46	21	15			
	2½				68	16	4	
	3½			54	43	7	1	
	4½			92	46	9	3	
	5½			69	48	6	1	
	6½			28	23	1		
	7½			9	15	3		
	8½			3	7	2		

Table 11. Humidity Readings at Each Hour of the Blooming Period of Paspalum sp. after Reversal to Its Normal Relation to Light with Temperature Constant (76°-80° F) after the Second Day

Day of bloom	4-5	5-6	6-7	7-8	8-9
A.M. 1	74	70	65	58	54
A.M. 2	78	72	70	64	60
P.M. 2½	54	58	64	68	72
P.M. 3½	54	56	60	66	69
P.M. 4½	58	60	66	70	74
P.M. 5½	50	52	58	62	66
P.M. 6½	54	56	60	66	70
P.M. 7½	50	54	58	62	66
P.M. 8½	54	60	66	72	74

ditions of relatively low humidity (Tables 1, 2 and 3) and under conditions of relatively high humidity (Table 10) shows that the effect of humidity within these ranges, upon flowering of these species was negligible.

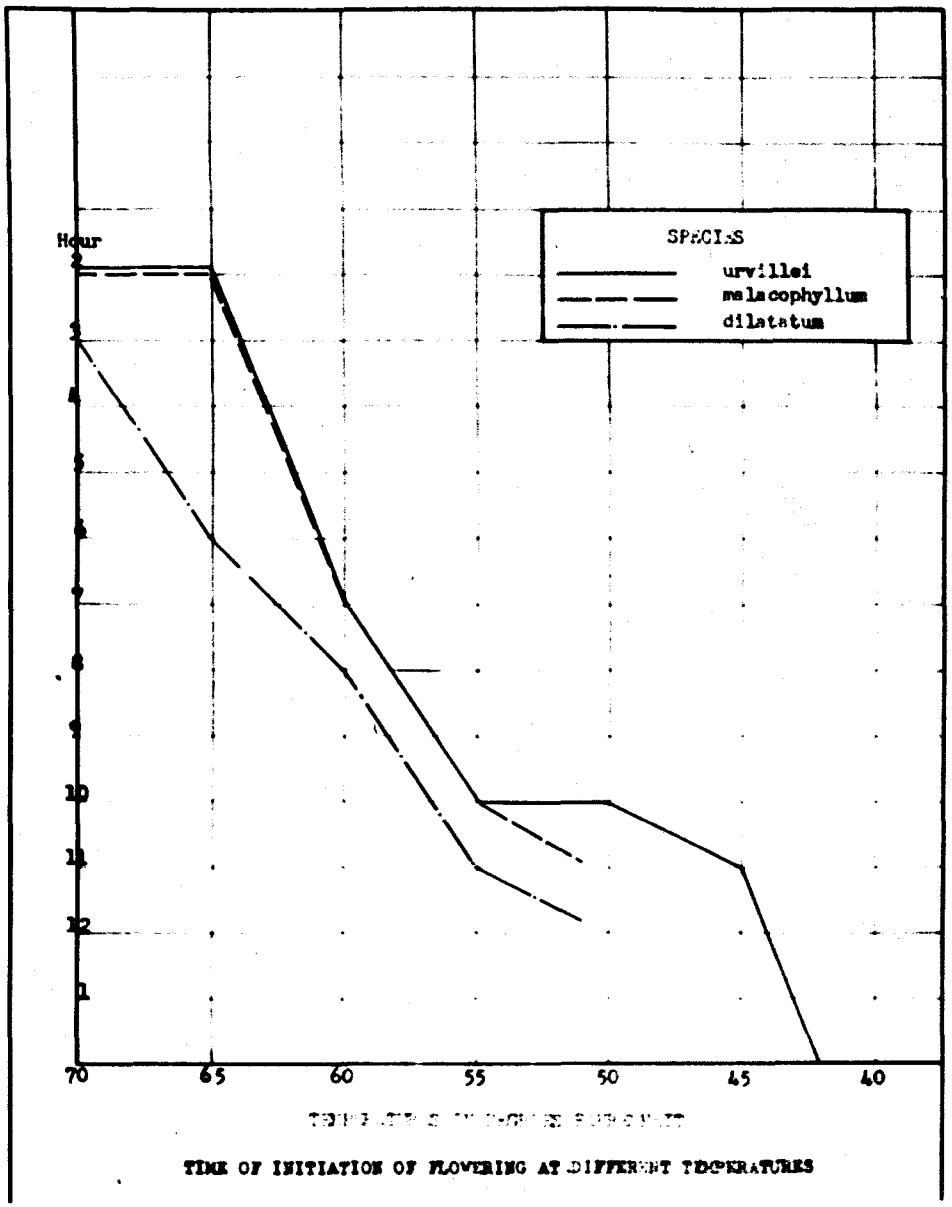
Paspalum malacophyllum did not bloom outside during rains but has been observed to bloom in the greenhouse during heavy rains. P. dilatatum and P. urvillei bloom during the rains but flowering of the individual floret proceeds at a reduced rate.

The flowering of the three species at constant humidity (70 per cent) and varying temperatures is given in Table 12, as the average of all counts for five days. The hour at which flowering began is shown by Figure 16. At 70° F. the three species began blooming at the same time as when under average summer conditions (Tables 1, 2 and 3). At 65° F. P. urvillei and P. malacophyllum maintained their normal rate and time of blooming. Blooming was delayed three hours in P. dilatatum and was quite irregular. Reducing the temperature to 60° F. delayed the beginning of blooming in all three species for five hours. A marked delay in the time required for the beginning of blooming in P. urvillei and P. malacophyllum occurred at this temperature.

A temperature of 55° F. further delayed blooming in all species eight hours. At 60° and 55° all species began blooming within the same hour. At the low temperature of 42° F. P. urvillei began blooming,

Table 12. Five-day Average of Flowering of Paspalum sp. at Constant Humidity and Varying Temperatures.

Species	Temperature ° F.	Time of flowering - hourly intervals									
		6-7	7-8	8-9	9-10	10-11	11-12	12-1	1-2	2-3	3-4
P. dilatatum	40	None									
	45	None									
	51							7	12	14	8
	55						4	10	11	21	
	60			6	11	26	2				
	65	15	34	17	2						
P. urvillei	40	None									
	45						4	6	8	9	
	51					2	10	13	18	7	
	55					2	12	15	10	6	
	60		6	11	15	13	7				
	65	Normal									
	70	Normal									
P. malacophyllum	40	None									
	45	None									
	51						8	19	24	3	
	55					13	19	18	27	1	
	60		4	7	15	23	18				
	65	Normal									
	70	Normal									



TIME OF INITIATION OF FLOWERING AT DIFFERENT TEMPERATURES

Figure 16.

whereas P. malacophyllum and P. dilatatum did not begin blooming at temperatures lower than 51° F. Plants of these species kept at temperatures lower than 51° F. for five days failed to bloom. However, if the plants which were exposed to temperatures below which they would not bloom, were exposed to higher temperatures in the presence of light, they responded immediately by blooming. Blooming may be induced at any desired time and at the same time in all species by the control of temperature and light.

In all cases in which blooming was delayed it was very irregular. The irregularity in blooming progressively increases as the temperature is reduced to the critical point at which blooming will not take place.

Tables 10 and 12 respectively show the lack of influence of humidity and the extreme importance of temperature and light upon the rate and time of blooming. The effect of temperature upon the time that the individual florets are open is of greater importance from a breeding standpoint than is the rate of blooming. The number of florets on each raceme, their irregular order of blooming, and their small size make emasculation very difficult.

The paired spikelets of these three species of Paspalum are crowded into four rows along the entire length of the raceme (Figure 7). The florets of the outer rows are slightly larger than those nearest the rachis (the inner rows). The outer florets of P. dilatatum average 3.39 long and 2.15 mm. wide as compared to 2.98 mm. and 2.04 mm. length and width of the inner florets. Outer florets of P. urvillei average

2.34 mm. long and 1.32 mm. wide as compared to the 2.11 mm. length and 1.20 mm. width of the inner rows. The florets of P. malacophyllum are the smallest with a length of 1.94 mm. and a width of 0.94 mm. for the outside florets, while the inner florets averaged 1.79 mm. long and 0.77 mm. wide.

Diagrams of the inflorescences are shown in Figure 7. The time required for the process of blooming in the individual floret was taken on 20 florets of each species for five days at each temperature interval. This gave the time required for blooming in 100 florets at each temperature interval. Intervals of 5° F. were used from 55° to 85° F. The time required for opening in all species was approximately the same except that the abrupt increase in the time required for blooming in P. malacophyllum and P. urvillei occurred at a temperature 5° F. lower than in P. dilatatum. The time required for the blooming of 100 florets of P. dilatatum at each temperature are shown in the following table:

Table 13. Time (minutes) Required for Blooming of 100 Florets of Paspalum dilatatum.

Temperature Degrees F.	Range	Mean	Standard error	Standard error difference
55	261-447	362.02	43.57	
60	164-348	260.00	39.24	
65	67-189	142.74	29.94	
70	47-104	77.01	18.54	
75	38- 98	64.49	8.63	
80	36- 68	48.68	6.77	
85	28- 44	38.35	3.90	6.938

From this table it may be seen that temperatures below 70° F. greatly affect the time required for the process of blooming. With increased temperatures the time required for blooming is reduced. Blooming at high temperatures requires much less time and there is less variation in time for blooming between florets. This table also shows that when the beginning of blooming is delayed (Figure 16) there is also an increase in the time required for the blooming of the individual floret.

The time of anther dehiscence is extremely important in emasculation. The anthers are not extruded until the floret is fully spread, but this occurs within five minutes after the glumes have become fully spread at 70° F. to 75° F. As the temperature rises the time of anther dehiscence is reduced. At 85° F. and above the anthers have dehisced before the floret has become fully opened. Considerable numbers of anthers dehisce between 80° and 85° F. before the floret opens but all dehisce before opening at 85° or above.

Reducing the temperature below 70° F. for P. dilatatum and below 65° F. for P. urvillei and P. malacophyllum also reduces the time anthers remain pendent without bursting or dehiscing. Anthers do not dehisce for six hours at 51° F., for four hours at 55° F. and one-half hour at 60° F.

Observations and counts have been made on several other species of Paspalum. P. notatum Flugge, has the shortest flowering period of any species noted. This species has only two racemes per inflorescence

with an average of 110 florets. Under conditions shown in Table 4 these florets opened between 6:00 a.m. and 7:00 a.m. Flowering was completed in two days. The anthers dehisce from the bottom as they are being extruded and the stigmas are pollinated as the floret is opening. This same condition exists in P. ciliatifolium Michx., another species with a short flowering period. P. boscanium Flugge flowers sporadically throughout the day and is further characterized by the short time the individual floret is open. P. pubiflorum Rupr., P. distichum L., P. floridanum Michx., P. longipilum Nash, and P. lividum Trin. are intermediate to P. malacophyllum and P. dilatatum in order and rate of flowering.

The information obtained in these studies of the factors controlling flowering, the development of the embryo, and pollen germination has made possible the hybridization of Paspalum dilatatum with P. urvillei and P. malacophyllum.

Potted plants of each species were allowed to complete the third day of flowering. The florets that had bloomed were removed that day from inflorescences that were to be used as the female parent. All plants were placed in the control room (55° to 60° F.) at 6:00 p.m. on that day. Artificial lighting began the next day at 6:00 a.m. All florets opening on plants to be used as the female parent were emasculated. The emasculated plants were removed to another room at approximately 80° F. The pollen plants were quickly placed next to

the plant to be pollinated and their flowering racemes placed in contact with the emasculated racemes. Anthers dehisced within 5 minutes after removal from the control room. Pollinated inflorescences were covered and the non-flowering spikelets were removed late that same afternoon.

Several interesting observations have been made on the F_1 of the hybrids produced. P. urvillei x P. malacophyllum; The germination of the seed produced by the F_1 obtained from these "high" seed producers was only 0.5 per cent. Plants produced from such seed were the same morphologically as the F_1 parent. Dominant characters from the urvillei parent were yellow anther color, ligule length, leaf sheath spotting, leaf width, plant color (light green), and purple stigma. Dominant characters from the malacophyllum parent were plant type, leaf sheath length and hirsute nature, floret and inflorescence shape and size, and glabrous spikelets.

P. urvillei x P. dilatatum; The dominant characters from the urvillei parent were the shape and size of the florets and inflorescence. Dominant characters from the dilatatum parent were anther and stigma color, glabrous leaf sheath, decumbent plant type, and length of culms.

P. malacophyllum x P. dilatatum; The dominant characters from the malacophyllum parent were the shape and size of the florets and inflorescence, and leaf sheath markings. Dominant characters from the dilatatum parent were anther and stigma color, decumbent plant type, length of culms, and glabrous leaf sheath.

Embryology of *Paspalum dilatatum*

Pollen studies have shown that the pollen grains germinate very soon after anther dehiscence. The rate of pollen tube growth within the style has not been determined due to difficulties encountered in clearing the feathery, much branched stigmas. Pollen tube growth is probably rapid, as the stigmas appear to be dry and dead within two hours after anther dehiscence. It has been noted that during periods of rainy weather, with the accompanying high humidity, some stigmas are fresh the next day after flowering. The pollinating of such stigmas with fresh pollen, however, has not induced fertilization. This indicates that fertilization occurs the day of flowering, possibly very soon after flowering. Delay in collecting pollen has resulted in a lowered pollen germination on artificial media.

The time interval between pollination and fertilization, under average summer conditions, is between 8 and 12 hours. Zygotes have been found as early as eight hours after pollination (Figure 17), and small proembryos after 12 hours (Figures 18, 19 and 20). Twelve hours after pollination the proembryo contains from two to eight cells and measures approximately 25 to 29 microns in length.

The fertilization of the egg and the polar nuclei by the male gamete takes place soon after the pollen tube enters the embryo sac. The zygote and primary endosperm nucleus undergo division very soon after fusion is completed (Figure 18). The two-celled embryo has a

PLATE II.

Figure 17. Zygote 8 hours after pollination x 360.

Figures 18, 19 and 20. Proembryos 12 hours after pollination x 360.

Figure 21. Proembryo 24 hours after pollination showing rounded nature x 275.

Figure 22. Proembryo beginning to lengthen 36 hours after pollination x 215.

Figure 23. Proembryo 48 hours after pollination. Note cellular endosperm x 225.

Figure 24. Proembryo 60 hours after pollination x 300.

Figure 25. Embryo 72 hours after pollination showing a preliminary step in initiation of the axis x 200.

Figure 26. Four-day old embryo. The posterior lobe has differentiated x 245.

Figure 27. Embryo 5 days after pollination showing distinct coleoptile primordia and plumule growing point. The radicle begins to differentiate at this stage x 200.

Figure 28. Embryo 6 days after pollination showing rapid growth of coleoptile and the distinct seedling leaf primordia x 100.

Figure 29. Mature embryo 18 days after pollination x 100.

Figure 30. Cross-section of a 6 day embryo showing seedling leaf and coleoptile around the plumule growing point x 115.

Figure 31. Cross-section of a 14 day old embryo showing the seedling leaf and the initiation of another leaf primordium x 90.

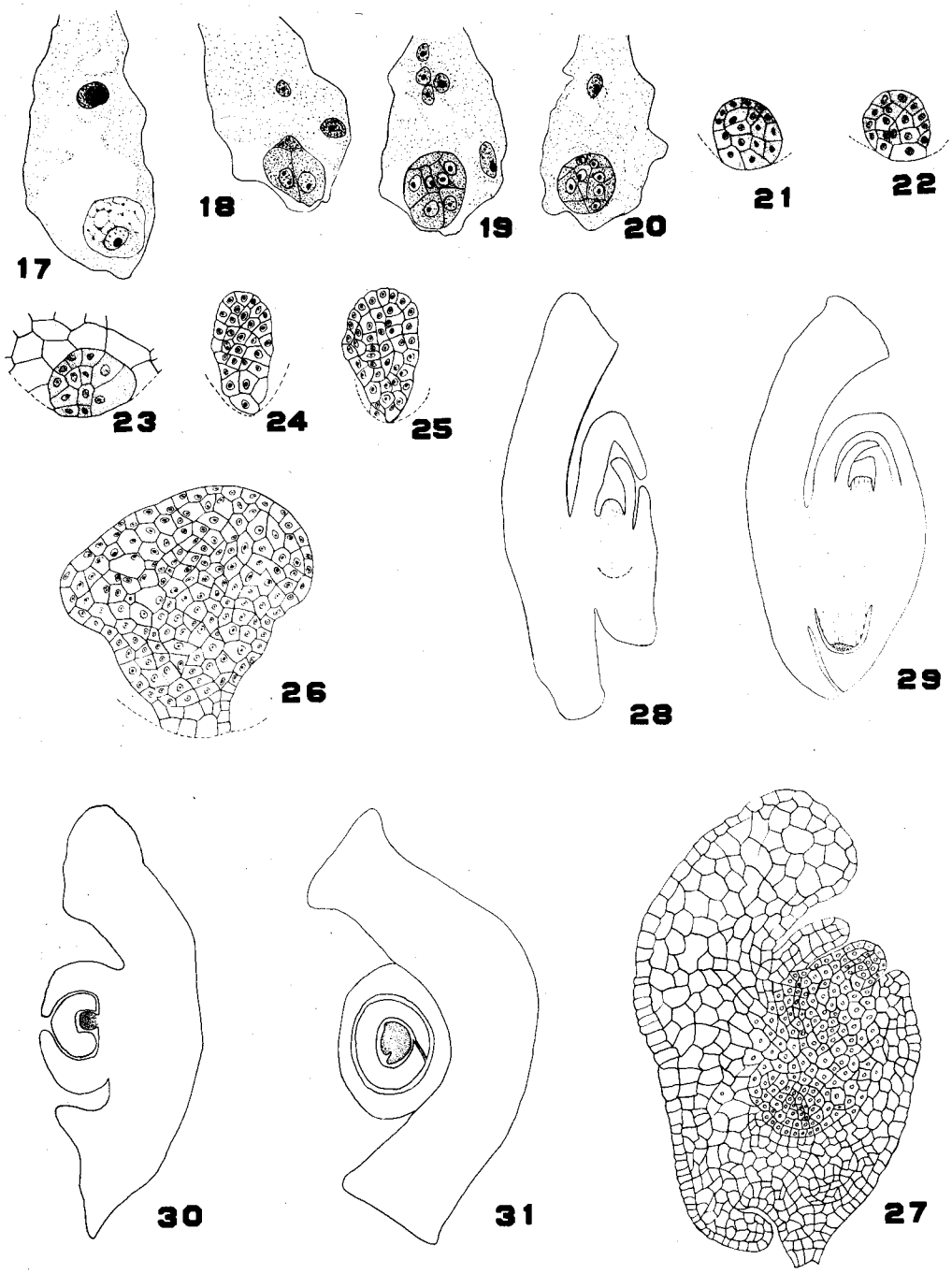


PLATE II.

Embryology of Paspalum dilatatum

small apical cell and a much larger basal cell. Rapid endosperm nuclear divisions occur, but the nuclei are free.

Twenty-four hours after pollination the proembryo becomes rounded (Figure 21). The difference between the small apical cells and the large basal cells is more pronounced than at the 12-hour stage. The endosperm nuclei continue to divide but are still free. Twenty-four hours after pollination the proembryo contains from eight to sixteen cells and ranges from 33 to 46 microns in length. The proembryo begins to lengthen at 36 hours. The apical cells continue to be smaller than the basal cells (Figure 22). The endosperm nuclei continue to divide without the formation of cell walls.

Further lengthening and enlargement of the proembryo is evident at 48 hours (Figure 23). The difference in the size of the apical cells and the basal cells becomes more pronounced. The endosperm begins to become cellular. At 60 hours (Figure 24) the embryo has lengthened to 56 microns, almost twice its width (32 microns). The apical cells of the club-shaped embryo continue to be much smaller than the basal cells. No organ primordia are evident at this stage.

Differentiation begins during the period from 60 to 72 hours. An increase in the rate of cell division in an anterior subapical region produces a slight protuberance (Figure 25). This evidently is a preliminary step in the initiation of the axis, and is not parallel with the axis of the proembryo. The embryo is approximately the same size as 12 hours before.

The apical region of the four-day old embryo is rounded. Meristematic activity on the posterior face of the embryo initiates the posterior lobe (Figure 26). The suspensor elongates and becomes sharply delimited from the body of the embryo. The differentiating axis (180 microns long) is now only slightly oblique to the longitudinal axis of the embryo, which is 148 microns long.

The differentiation and growth of the embryo is extremely rapid during the fourth to the fifth day (Figure 27). The large scutellum is derived from the posterior lobe and the distal lobe. The growing point of the plumule and the coleoptile primordia are very distinct. The coleoptile is formed as a ridge of tissue which nearly surrounds the central growing point. The definite arc-like arrangement of cells in the lower portion of the axis marks the beginning of the differentiation of the radicle. The embryo at this stage has elongated to approximately 342 microns.

On the sixth day the coleoptile has grown around toward the anterior side and is almost closed (Figure 28). The seedling leaf primordium has become quite distinct. The seedling leaf arises below the growing point as a ridge of tissue similar to and opposite that of the coleoptile primordium. Pronounced differentiation of the scutellar trace is now taking place. Within the root primordium certain cells have assumed a definite orientation which marks the delimitation of the coleorhiza and primary root. The scutellum has lengthened to 629 microns.

The coleoptile has closed by the seventh day. The side next to the scutellum grew faster than that on the anterior side so that the coleoptile pore is located on the anterior side of the coleoptile. The leaf primordium has enlarged. The cotyledonary node and coleorhiza are more pronounced. The coleorhiza was differentiated from the lower part of the embryo by the development of the primary root primordium. Differentiation of the root cap has begun. The embryo has now reached a length of 684 microns.

By the eighth day the leaf primordium has started to lap over the growing point and the coleorhize is very distinct; the scutellar trace has reached one-half the length of the plumule by the eleventh day. The leaf continues to lengthen. There is an overall growth and the embryo is practically mature in size. A mature embryo has a scutellum length of approximately 780 microns, a plumule-radicle axis length of 530 to 590 microns, and a depth of approximately 250 microns (Figure 29).

The seedling leaf continues to lengthen and is curled around the growing point at 16 days (Figure 31). Another leaf primordium has begun to differentiate which results in a small protuberance at the base of the growing point and opposite the primordium of the first leaf. This second leaf fails to develop further, as there is only one well-formed leaf in the mature embryo.

The relative position of the axis of the mature embryo changes slightly in the later stages of maturity. The axis is at first very

oblique and later assumes a position more nearly parallel to the main body of the embryo. However, at maturity it is still slightly oblique.

Chromosome Counts

Mitosis is most easily observed in the root tips. Somatic chromosomes at metaphase are predominantly V and J shaped, with the majority having a median spindle fiber attachment.

Mitosis appears to be rather regular in the three species and in the hybrids P. dilatatum x P. urvillei and P. dilatatum x P. malacophyllum. No lagging or other irregularity has been noted.

The chromosome number was determined in the three species and their hybrids. These counts show 20 to be the haploid (n) number.

Pollen Germination

Preliminary treatments administered to pollen of Paspalum dilatatum gave some information as to technic. It was found that in spite of low germination, agar was a better subtratum for germination than gelatine, gelatin-agar, blotting paper, or gum arabic. Any concentration of gelatin added to agar reduced pollen germination. Germination was progressively better on maltose, dextrose, levulose, and sucrose. Levulose and sucrose gave approximately the same germination. Humidities higher than 85 per cent resulted in clumping of pollen which made the counting difficult. Germination in water and sucrose solutions came as a surprise in view of the fact that there was considerable bursting of pollen.

The time of collecting pollen for testing proved to be of extreme importance. Pollen dusted from anthers one-half hour after dehiscence failed to germinate. Readings taken at intervals of one-half, 1, 2, 4, 8, and 24 hours after placement have shown that there is no increase in germination after one hour.

The results of pollen germination of P. dilatatum were very discouraging. The average percentages of germination on each medium, under conditions of constant pH or sugar concentrations and varying temperatures, are given in Table 14. The simple average of all germination percentages will show that, under these conditions, the germination of pollen on artificial media from a "good" seed producing selection (56-17) was no better than that from a low seed producing selection (11-48).

Averages given in Tables 15 and 16 show no significant differences between sugar concentrations, degree of acidity, or solution or agar media. Germination was slightly better on agar than in solutions. Variations between duplicate treatments were great. A certain concentration would yield good germination one day and none when repeated the next day.

Observations of the data indicate that temperatures of 30° C. and below are best for germination on artificial media. Good germination, however, has been obtained at higher temperatures.

The analysis of variance was made of pollen germination for four studies. The coefficients of variability ranged from 51 per cent to

Table 14. Average Per cent Pollen Germination of Two Selections of Paspalum dilatatum in Solution and Agar Media at Temperatures from 24 to 39° C.

Selection no.	Media	Per cent pollen germination at temperatures in degrees centigrade										
		24	27	29	30	31	32	33	34	37	39	Average
11-48	Solution pH 5.8	2.76	3.55	2.99	3.40	2.44	2.95	3.81	3.20	2.54	2.59	3.00
"	" 15% sucrose	2.96	4.25	4.11	2.65	4.06	2.45	3.58	3.02	3.30	2.42	3.28
"	Agar pH 5.8	3.08	3.85	4.14	3.70	3.46	4.17	2.97	3.58	2.17	3.91	3.50
"	" 15% sucrose	3.07	4.25	5.55	5.19	4.52	4.17	3.45	4.08	5.17	3.47	4.29
												3.52
56-17	Solution pH 5.8	4.16	4.54	3.61	3.74	3.64	3.72	3.48	2.76	2.94	2.48	3.51
"	" 15% sucrose	3.94	3.12	3.49	2.90	4.56	2.88	3.92	3.01	4.08	2.64	3.45
"	Agar pH 5.8	3.74	3.11	3.21	3.60	3.87	4.93	3.36	3.72	3.09	2.86	3.55
"	" 15% sucrose	2.38	4.53	5.74	6.22	5.52	3.36	3.41	3.05	3.86	3.08	3.92
												3.62

Table 15. Average Per cent Pollen Germination of Two Selections of *Paspalum dilatatum* in Solution and Agar Media at Sucrose Concentrations from 0.0 to 40.0 Per cent.

Selection no.	Media	Sucrose concentration in per cent									
		0.0	5.0	10.0	15.0	20.0	25.0	30.0	35.0	40.0	Average
11-48	Solution pH 5.8	3.05	3.20	3.40	3.59	3.67	3.30	2.11	2.37	2.12	2.98
"	Agar pH 5.8	3.78	3.95	4.09	4.34	4.29	3.48	4.28	2.59	3.80	3.84
56-17	Solution pH 5.8	3.68	3.58	4.53	3.87	2.91	2.61	3.55	3.68	3.14	3.55
"	Agar pH 5.8	3.52	4.04	3.93	3.92	4.06	4.00	2.63	2.73	2.98	3.53

Table 16. Average Per cent Pollen Germination of Two Selections of *Paspalum dilatatum* in Solution and Agar Media of pH from 4.4 to 7.6.

Selection no.	Media	pH of Media									
		4.4	4.8	5.2	5.6	6.0	6.4	6.8	7.2	7.6	Average
11-48	Solution 15% sucrose	3.53	3.20	3.67	3.20	3.35	3.57	3.09	2.91	3.17	3.30
"	Agar 15% sucrose	3.84	4.49	4.68	3.99	4.09	4.69	4.56	4.22	3.87	4.27
56-17	Solution 15% sucrose	3.04	2.85	3.42	3.40	3.83	3.25	3.60	3.83	3.14	3.37
"	Agar 15% sucrose	4.49	4.94	4.52	3.80	4.18	3.93	3.88	3.82	3.51	4.12

79 per cent which shows an extremely large sampling error. From

Table 17. Analysis of Variance on Pollen Germinated at Temperatures from 24 to 39° C. and pH from 4.4 to 7.8 with Agar and Sucrose Constant.

Variation due to	D.F.	M.S.	F
Total	359	-	
Total plants	179	-	
pH	17	1.90	-
Temp.	9	24.68	3.03**
pH x Temp.	153	3.16	-
Sampling error	180	8.14	

inspection of the data, in relation to the high error, it did not seem necessary to complete the analysis for all conditions under which pollen germinations were made. Temperature relations were found to give significant differences in two studies. The analysis of variance in Table 17 is for one of the experiments in which temperatures were varied from 24° to 39° C. and pH from 4.4 to 7.8. Significant differences at the one per cent level were obtained for temperatures, but the differences due to varying pH and the interaction of pH and temperature were not significant when tested against sampling error.

Pollen size varied very little. The spherical pollen grains of P. dilatatum averaged 34.7 microns, those of P. urvillei 33.6 microns, and those of P. malacophyllum 33.4 microns.

Selection 56-17, in years of light ergot infection, has continued to produce a higher percentage of good seed than selection 11-48 (15). The per cent of pollen germination on artificial media obtained under the conditions of temperature, pH, and sucrose concentrations used show that this cannot be used as a method of selection for seed production.

DISCUSSION

Flowering

The flowering experiments show rather definitely that light, or the absence of light, and temperature exert the principal external influences on the periodicity of flowering. Light, below a rather definite temperature, failed to induce flowering (Figure 16 and Table 12), indicating that temperature is the more important factor. The increase in time required for flowering of the individual floret due to reduced temperatures tends to verify this assumption. Sando (91), Li, et al. (50), Stephens and Quinby (85) Miscnoe (57), and Rangaswami (69) showed that lowering the temperature reduces the rate of flowering. Only Wolfe (96) has given the minimum temperature at which a grass will bloom. Experience with these species also tends further to verify this assumption. After a sudden drop in temperature or in late fall, when temperatures are low, flowering is greatly delayed. P. dilatatum has been observed to flower as late as 1:00 p.m. in late October. The sharp reduction in the time required for flowering (Table 13) of the individual floret also emphasizes the influence of temperature and indicates that temperature is the more important factor within a rather definite range (Tables 12 and 13).

Placing plants in a dark room during the day and exposing them to artificial light at night and reversing their normal rhythm (Figures 12,

13 and 14) shows that light conditions are also a factor in governing the time of flowering. The flowering of P. malacophyllum (Figure 14) in darkness after day reversal, even on the day of the change, indicates that temperature is the more important factor, although light did change its flowering rhythm. The more normal flowering of P. urvillei shows this species to be slightly more sensitive to light and four days are necessary before it regains its flowering (before light). The sudden rise in flowering of P. dilatatum (Figure 12) after lighting shows the influence of light on this species. A comparison of Figures 12, 13 and 14 indicates a considerable difference between these species. The increase in the amount of light necessary to induce flowering at reduced temperatures (Figure 16, Table 12) and the necessity of light for flowering at considerably higher temperatures (Figure 12) suggests that different physiological processes may control the flowering in the different species. The relative proportion of light and darkness in the reproduction processes of these species should be investigated. The minimum amount of darkness needed before the flowering process can be affected by light should also be studied. It might be possible to cause two periods of flowering in one 24-hour period and bring the periods of flowering of the different species together. Burton (23) delayed flowering in P. dilatatum by enclosing panicles in light-proof bags but could not obtain normal flowering later in the day when temperatures were higher. He reported observations without

counts or measurements. He also reported that flowering would not take place in darkness but that some flowers bloomed in the light-proof bags.

The effect of humidity within the ranges used seems to be negligible. P. malacophyllum flowered at high humidities but would not flower during a rain. Burton (23) found no blooming in this species during a rain, either in the greenhouse or in the rain. Inasmuch as soil moisture (Figure 11 and Table 6) or reversing the natural relation of light and darkness to temperature and humidity (Table 8) failed to affect flowering in this species, it would appear that the falling of rains or free water exerts a physical effect upon flowering. The fact that most species of Paspalum are usually found in low bottoms and along streams where humidity is unusually high during the hours of flowering and most species bloom during rain, would tend to verify the assumption that the effect of humidity upon flowering is negligible. Light and temperature are both abnormal during damp or rainy weather and effects ascribed to humidity may be due to the effects of other factors. The lack of influence of humidity on the flowering of Paspalum sp. has been found true with other species of grasses (34, 57, 85, 96).

The present studies show that, within a definite temperature range, and under a normal diurnal alternation, temperature is the most important factor. Light, or periods of light and darkness, appears to be an important factor secondary to temperature.

Embryology

Following the division of the zygote, the subsequent planes of cell division in the proembryo are irregular and it would appear that no special significance can be attached to the sequence of cell divisions nor to the arrangement of the cells in the early development of the embryo. This is in agreement with the findings of Randolph (68) with corn and Merry (56) with barley. It indicates that the factors controlling the growth of the proembryo for the first 60 hours affect the embryo as a whole rather than definite cells. Souèges (83) stated that the parts of the embryo are already determined in particular cells in the 16-cell embryo. Norner (61) also attempted to classify the arrangement of the cells according to the manner in which they divided.

The proembryo is almost round for the first 60 hours after pollination and is comparable to the same stages of growth in Eragrostis ciliianensis (All.) Link as described by Stover (87). During these stages there is no differentiation except for the difference in the size of the apical and basal cells. This may suggest a gradient from the apex to the base of some factors which may control the rate of division of the cells. The appearance of smaller cells in certain parts of the embryo at three and four days would indicate that such or similar factors had become concentrated in those regions.

The development of the embryo from the initiation of the axis

(3 days) to maturity follows that of the ordinary grass type embryo as described by LaRue and Avery (48), Randolph (68), Merry (56), Stover (87), and summarized by McCall (54).

The mature embryo of P. dilatatum differs from the embryos of many grasses in that it has only one foliage leaf and no epiblast. It differs from the embryo of P. pubiflorum glabrum (43) in that the axis is more oblique to the main body of the embryo.

The embryo of P. dilatatum is mature at 14 to 18 days after pollination. This is relatively rapid in comparison with the development of the embryo in maize, which requires 45 days (68) and 35 days (56) for that in barley. The rate of maturity is slightly faster than that found for Zizania aquatica by LaRue and Avery (48) who regarded the 19-day-old embryo to be mature, but the seed "unripe". The fastest rate of development of a grass embryo is that reported by Jenkins (41) for Poa annua in which 82 per cent of the caryopses were mature fourteen days after pollination. Observations have shown that seed from the topmost raceme of P. boscanium are shattering before the florets of the lowest raceme have finished flowering.

Some evidence of cleavage polyembrony has been found in P. dilatatum, which might well be one cause of the low percentage of seed set.

Chromosome Numbers

The chromosome numbers found verify the counts of Marchall (53), Church (26), Krishnaswamy (47), Saura (75), and Burton (22) that 20 is

the haploid (n) number in these species. No evidence of divergence from this number was found in these species or their hybrids, unlike other species reported by Avdulov and Titova (6).

There seems to be no correlation between the chromosome number, chromosome morphology, and morphological characters of these species. The cause of morphological differences between these species must be due to hereditable characters. Species of the genus Paspalum appear to intergrade (24), whether by hybridization or by reverting to ancestral form, and the species appear to be closely related. The evident taxonomic relationships and the existence of polyploidy within this genus might suggest the presence of genomes. However, taxonomic studies do not indicate any definite descent.

Pollen Germination

Although the per cent of pollen germination on artificial media was extremely low (Table 14), several characteristics of pollen of P. dilatatum were noted and pollen studies with other species of grass were verified.

Pollen of this species is very short lived. No germination was obtained with pollen one-half hour after dehiscence. This short-lived characteristic of grass pollen has been noted by Anthony and Harlan (2) in barley, Bair and Loomis (9) in corn, Mercado (55) and Sartoris (73) in sugarcane, and Reyes (70) in rice. Summaries of pollen longevity by Holman and Brubaker (39) and Doroshenko (29) also show that the pollen

of the Gramineae has a very short life span. Pollen in other species at one hour after dehiscence has been called "old" by Smith (81).

Exact concentrations for best germination are difficult to ascertain. The large experimental error shows wide differences with the same concentration. This is in agreement with most investigators who have found that results with grass pollen were not constant. At times the germination from a certain concentration was excellent, and on repetition the same strength failed to induce germination. Doroshenko (29) shows that concentrations vary not only with species but from year to year. Smith (81) reports differences between anthers of the same flower, between flowers of the same plant, and between plants. The results obtained in these experiments certainly bear out the fact that the pollen of grasses is difficult to germinate and that the variability is great.

It is possible that the sucrose concentration used was not great enough. Doroshenko (29) reports that Poa chaixi required a sucrose concentration of 50 per cent.

Further work should be done with growth promoting substances inasmuch as excessive concentrations were used in the present experiments (81). Plant parts should also be included in the media.

The low germinations obtained were probably not due to defective pollen as measured by the criterion used (95). Shrunken or "defective" pollen ranged from 5 to 37 per cent in 56-17, from 0 to 43 per cent

in 11-48, 12 to 36 per cent in P. malacophyllum, and 7 to 30 per cent in P. urvillei. However, relatively high percentages of pollen germination (25 per cent) were obtained with the two latter species.

The immediate germination of pollen on the stigma before desication or pollen bursting probably accounts for the seed setting in these species. Lower temperatures at flowering and a higher percentage of good pollen probably accounts for the higher percentage of seed setting in P. malacophyllum and P. urvillei.

SUMMARY

Species of Paspalum vary in the time of flowering under normal environmental conditions. The peak of flowering in P. dilatatum is two hours after the maximum of P. urvillei and three hours after the maximum of P. malacophyllum. The peak of flowering is reached in a given inflorescence the fourth day in P. dilatatum, the fifth day in P. malacophyllum, and the eighth day in P. urvillei.

The three species have the same order of flowering, from the top raceme down the inflorescence and from the end-most spikelet back toward its base. The time required for all florets of the inflorescences to complete blooming varies with the size of the inflorescence. The florets of the paired spikelet usually flower on alternate days.

The effect of humidity, within the temperature ranges used, was found to be negligible.

Soil moisture has little effect upon the rate or order of flowering.

The time required for opening and closing of florets and anther dehiscence varies inversely with the temperature.

Reversing the normal alternation of light and darkness reverses the normal periodicity of flowering. The order of flowering under reversed alternation of light and darkness is the same as that under normal conditions. Light is also a factor governing the time of flowering, but light is secondary to temperature.

Flowering may be induced at any desired time and at the same time in the three species by the proper manipulation of temperature and light. The experimental control of flowering has made possible the hybridization of P. dilatatum with P. urvillei and P. malacophyllum.

The time interval between pollination and fertilization of P. dilatatum, under average summer conditions, is between 8 and 12 hours.

The endosperm nuclei are free for the first two days. The embryo contains from 16 to 48 cells. There is no consistent arrangement or zonation of cells in the proembryo.

Initiation of the coleoptile-radicle axis begins from 60 to 72 hours. The development of the embryo is extremely rapid from the fourth to the eighth day and is structurally complete in 14 to 18 days. The mature embryo has one foliage leaf and a distinct radicle with a root cap. There is no epiblast.

The diploid chromosome numbers in the three species used is 40.

The pollen of P. dilatatum is short-lived. Widely varying percentages of pollen germination were obtained. Temperatures of 30° F. and below appear to be optimum.

Artificial pollen germination was found to be unreliable as a basis for seed selection in Paspalum dilatatum.

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